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MICROBIOLOGICAL TRANSFORMATIONS OF FLAVONOIDS—II

TRANSFORMATIONS OF (±) FLAVANONE*

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Abstract—From the incubation of (±) flavanone (I) with *Gibberella fujikuroi* a number of products *viz.* (−) flavan-4α-ol (II), 2′-hydroxychalcone (III), 2′,4-dihydroxydihydrochalcone (IV), 2′,4-dihydroxychalcone (V), (±) 4′-hydroxyflavanone (VI) and (−) 4′-hydroxyflavan-4α-ol (VII) have been isolated and characterized.

IN A previous publication[1] we described the isolation and characterization of a neutral product (II) from the microbiological transformation of flavanone using *Gibberella fujikuroi* as the organism. We now wish to report the isolation and characterization of a few additional products from the same experiment.

The extracts from mycelia and the filtrates were fractionated into neutral and acidic portions. From the neutral portion flavanone (I) (unchanged substrate) and (−) flavan-4α-ol (II)[1] were isolated. The acidic portion on chromatography over silica gel gave five fractions (A, B, C, D and E), from which the following compounds were obtained:

From fraction A, a yellow product was isolated which was characterized as 2′-hydroxychalcone (III) on the basis of colour reactions and spectral data and by direct comparison with an authentic sample.[2]

Fraction B gave a colourless compound $C_{15}H_{14}O_3$. It showed the presence of OH, CO and 1,4-disubstituted aromatic absorptions in the IR spectrum. In the UV spectrum a pronounced absorption max was observed at 252 nm. The presence of a chelated OH group was indicated on the basis of a positive ferric chloride reaction and a bathochromic shift in the UV absorption max on the addition of $AlCl_3$. In the NMR spectrum, of the 8 protons appearing in the aromatic region between τ 2·1 to 3·4, four formed an A_2B_2 system (τ 2·82, $J = 9$ c/s and τ 3·19, $J = 9$ c/s). Two singlets (exchangeable with D_2O) at $\tau - 2{\cdot}37$ and τ 5·05 were attributed to two OH groups, the former being a chelated OH group. A 4-proton multiplet centered at τ 6·84 could be assigned to the protons of two methylene groups. On the basis of the spectral and chemical data the compound was characterized as 2′,4-dihydroxydihydrochalcone (IV).[3] This structure was confirmed by a direct comparison with a synthetic sample of IV prepared by the selective reduction of 2′,4-dihydroxychalcone.

From fraction C, a yellow product $C_{15}H_{12}O_3$ was obtained and the colour reactions indicated it to be a chalcone or an aurone. The UV spectrum (absorption maxima at

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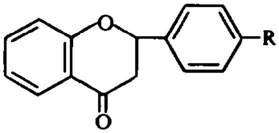
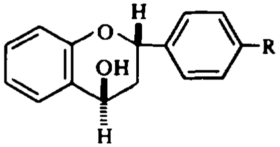
* Microbiological Transformation of Flavanone, Tetrahedron Letters, 4003, 1968, forms Part I of this series.

368 and 241 nm; log ε, 4·45 and 4·08), however, favoured a chalcone structure.[4] A chelated OH group was found present. The IR absorption at 823 cm^{-1} indicated the presence of a 1,4-disubstituted aromatic ring. The above data suggested the product to be 2′,4-dihydroxychalcone (V), which was confirmed by a direct comparison with an authentic sample.[5]

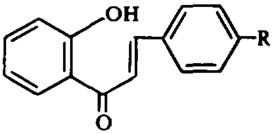
Fraction D gave a colourless compound, $C_{15}H_{12}O_3$. The IR spectrum showed absorptions for OH and CO groups. The OH group was phenolic (compound soluble in sodium hydroxide solution) but was not chelated. The colour reactions suggested it to be a flavanone.[6] The UV spectrum showed prominent absorption at 251 nm (log ε, 4·00) and an inflection at 319 nm (log ε, 3·54), thus supporting a flavanone structure. The NMR spectrum showed a quartet at τ 4·45 (1 H) due to the benzylic proton at position-2. Two pairs of doublets (τ 3·04, $J = 8$ c/s and τ 2·53, $J = 9$ c/s) represent an A_2B_2 system suggesting a 4′-substituted flavanone which was further supported by an IR absorption at 828 cm^{-1}. As expected for a 4′-hydroxyflavanone,[7] the compound VI was found to undergo a facile ring opening to the corresponding chalcone ($\lambda_{max}^{CH_3OH-NaOH}$ 429 and 264 nm). The compound was found identical (i.r. UV and NMR) with an authentic sample of (±) 4′-hydroxyflavanone (VI)[5].



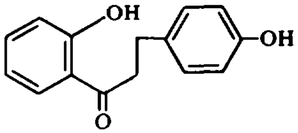
I: R = H; VI, R = OH



II; R = H; VII, R = OH



III: R = H; V, R = OH



IV

Of the products isolated so far the most polar compound, $C_{15}H_{14}O_3$ was obtained from fraction E which was found to be optically active. It dissolved in sodium hydroxide solution. The IR spectrum of the compound showed the OH and aromatic absorptions and absence of a CO group. It gave intense purple colour with conc sulphuric acid and exhibited UV maxima at 225 (log ε, 4·25), 275 (log ε, 3·52) and 281 nm (log ε, 3·47). The above data indicated a flavan-4-ol[1, 8] structure for the compound. The phenolic OH group was assigned 4′ position on the basis of IR spectrum (absorption at 831 cm^{-1}). The NMR spectrum of the compound also supported the above assignments. The protons of the aromatic rings A and B appeared between τ 2·6 to 3·3. The protons of the ring B gave A_2B_2 type signals at τ 2·65 (d, $J = 9$ c/s) and τ 3·12 (d, $J = 9$ c/s). The proton at position 2 appeared as a quartet at τ 4·77 ($J_{2,3a} + J_{2,3e} =$ 14 c/s). The H-4 proton gave a triplet at τ 5·21 ($J_{4,3a} + J_{4,3e} = 6$ c/s). These data indicate that H-2 and H-4 are *axial* and *quasi equtorial* and hence should be *trans* to each other.[9, 10] An authentic sample of (±) 4′-hydroxyflavan-4α-ol was prepared from (±) 4′-hydroxyflavanone by Al/Hg reduction.[11] A comparison of IR, UV and NMR spectra with the authentic sample together with the optical rotation data confirmed the identity of the compound as (−) 4′-hydroxyflavan-4α-ol (VII).

DISCUSSION

An examination of the transformation products discussed above clearly indicates that aromatic hydroxylation of B-ring is an important reaction leading to the formation of IV, V, VI and VII. The formation of dihydrochalcone (IV) must have resulted from the corresponding chalcone (V). Whether the formation of V from VI and of III from I are simple chemical equilibrations or instead enzymatic reactions is not yet clear. The products II and VII represent stereospecific reductions of the CO group at C-4 in the corresponding flavanones (I and VI). Similar 'product stereospecificity' has been discussed by Prelog in the microbiological reduction of α-decalones.[12] It is hoped that by the use of suitably selected flavonoids, microbiological transformations may provide a useful route to the formation of some optically active compounds in the series. Work on the rates of formation and utilization of some of the above products is likely to throw light on the pathway(s) of these transformations, particularly where more than one reaction are involved. Such work is in progress and will be reported later.

EXPERIMENTAL

M.ps are uncorrected. UV spectra were taken on a Zeiss RPQ 20A recording spectrophotometer. IR spectra were taken on a Perkin Elmer Infracord 137-B spectrophotometer. NMR spectra were determined with a Varian A-60A spectrometer. Optical rotations were determined with a Hilger-Watt polarimeter.*

Six-day old culture of *Gibberella fujikuroi*† grown in modified Czapek Dox medium[13,14] was used in the experiments. In the preliminary experiments, five graded concentrations (0·01 to 0·05%) of I were used. Concentration of 0·03% of the substrate was found to be optimal. The fermentation was carried out with intermittent aeration using 800 mg/3 l. of medium of substrate, for 20 days after which the mycelia were separated by filtration and extracted in a Waring blender with acetone. The concentrate after removal of acetone was extracted with EtOAc. The filtrate was separately extracted with $CHCl_3$. A TLC examination (SiO_2 gel G; E. Merck, benzene–EtOAc, 100:20) of extracts from the mycelia and the filtrate indicated the presence of identical components in both. However, the major part of the extractive was obtained from the mycelia. Both the $CHCl_3$ and EtOAc extracts were combined and fractionated into neutral and acidic portions by extracting with 1N NaOH.

Neutral fraction. The neutral portion was fractionated by chromatography (SiO_2 gel, E. Merck less than 0·08 mm/hyflosupercel 8:2). From the initial benzene eluate, a colourless crystalline solid (280 mg), m.p. 75–76° was obtained, which was identified as I (unchanged substrate). Compound II was obtained on further elution with benzene and is discussed below:

(−) *Flavan-4α-ol* (II). It crystallized from benzene–pet. ether as colourless needles (15 mg) m.p. 127° $[\alpha]_D^{25°}$ − 27·3° (*c*, 1·4, $CHCl_3$); UV absorption, $\lambda_{max}^{CH_3OH}$, 277 and 284 nm (log ε, 3·35 and 3·31 respectively); IR (KBr) 3436, 1616, 1587, 1488 and 1238 cm^{-1}; NMR in $CDCl_3$: τ 7·7 (m, H_{3a} and H_{3e}), τ 4·7 (q, $J_{2,3a} + J_{2,3e} = 15$ c/s; H_2) τ 5·15 (t, $J_{4,3a} + J_{4,3e} = 6$ c/s; H_4), τ 2·5–3·25 (complex m, aromatic protons). (Found: C, 80·0; H, 6·6 Calc. for $C_{15}H_{14}O_2$: C, 79·7; H, 6·2%). Compound II does not give ferric reaction. It gave red colour with conc. H_2SO_4 or HCl.

Acidic fraction. The acidic fraction was chromatographed over silica gel (E. Merck, less than 0·08 mm and hyflosupercel mixture 8:2) and eluted with benzene–EtOAc containing increasing proportions of EtOAc, when five fractions A, B, C, D and E were obtained.

Fraction A—2'-hydroxychalcone (III). The product obtained from fraction A crystallized as yellow needles from benzene-pet. ether (3 mg) m.p. 88–89°. It gave brown ferric reaction and dissolved in NaOH aq

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† *G. fujikuroi* was obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Poona.

giving deep yellow soln; $\lambda_{max}^{CH_3OH}$ 221 and 314 nm (log ε, 4·07 and 4·3). It was identified as 2′-hydroxychalcone which was confirmed by comparison with a synthetic sample.[2]

Fraction B—2′,4-dihydroxydihydrochalcone (IV). A colourless crystalline solid was obtained from the fraction B using benzene–pet. ether as solvent for crystallization (8 mg), m.p. 106–107°. (Found: C, 74·4; H, 6·1. Calc. for $C_{15}H_{14}O_3$ C, 74·4; H, 5·8%). It gave brown colour with alc. $FeCl_3$. It dissolved in NaOH aq and conc H_2SO_4 to give pale yellow solns. Addition of $AlCl_3$ soln to an alc. soln of IV gave a yellow soln which fluoresced green in UV light. A spot of the compound on a TLC plate appeared yellow in UV light but became intense blue on heating to 100° for 3 min. UV absorption: $\lambda_{max}^{CH_3OH}$, 252, 278 and 324 nm (log ε, 4·04, 3·37 and 3·62 respectively), $\lambda_{max}^{CH_3OH-AlCl_3}$, 273, 312 (sh) and 382 nm, IR (KBr), 3436, 1639, 1616, 1587, 1517, 1495, 1203, 829, 762 and 728 cm^{-1}; NMR data ($CDCl_3$): two doublets at τ 2·82 and τ 3·19 ($J = 9$ c/s) represent 2, 6, 3 and 5 protons, τ 6·84 (m, 4H) are due to two methylene protons and τ—2·37 and τ 5·05 (exchangeable with D_2O) represent OH protons at 2′ and 4 positions respectively.

Synthetic sample of 2′,4-dihydroxydihydrochalcone was obtained by the selective hydrogenation of 2′,4-dihydroxychalcone (5% Pd/C, 0·5 hr, EtOH), m.p. 106°. (Found: C, 74·1; H, 5·7 Calc. for $C_{15}H_{14}O_3$ C, 74·4; H, 5·8%).

Fraction C—2′,4-dihydroxychalcone (V). Fraction C, on crystallization from benzene–pet. ether furnished a yellow crystalline solid (144 mg), m.p. 157–158°. (Found: C, 75·3; H, 5·2. Calc. for $C_{15}H_{12}O_3$: C, 75·0; H, 5·0%). It gave orange soln on addition of conc H_2SO_4, NaOH aq and $SbCl_5$ (in CCl_4). It did not answer Mg–HCl reduction test; however, on reduction with $NaBH_4$ followed by acidification, a pink coloration developed. It gave brown colour with alc. $FeCl_3$; IR (KBr): 3378, 1637, 1613, 1582, 1513, 1490, 1208, 823 and 751 cm^{-1}; UV absorption: $\lambda_{max}^{CH_3OH}$, 241 and 368 nm (log ε, 4·08 and 4·45). $\lambda_{max}^{CH_3OH-NaOAc}$, 271 and 435 nm $\lambda_{max}^{CH_3OH-AlCl_3}$ 288 and 442 nm, $\lambda_{max}^{CH_3OH-NaOCH_3}$ 271 and 438 nm and $\lambda_{max}^{CH_3OH-NaOH}$ 240 and 412 nm.

Fraction D—(±)4′-hydroxyflavanone (VI). Fraction D, on evaporation left a solid which on crystallization from EtOAc–pet. ether furnished colourless prisms (20 mg) m.p. 189–190°. (Found: C, 75·3; H, 5·3. Calc. for $C_{15}H_{12}O_3$, C, 75·0; H, 5·0%). It gave orange colouration with conc H_2SO_4 and NaOH aq and yellow with SCl_5. Reduction with Mg–HCl gave red colour while $NaBH_4$–HCl gave pink colour. UV absorption: $\lambda_{max}^{CH_3OH}$ 251 and 319 nm (log ε, 4·00 and 3·54 respectively); $\lambda_{max}^{CH_3OH-AlCl_3}$ 252 and 318 nm $\lambda_{max}^{CH_3OH-NaOH}$ 429 and 264 nm; NMR data (acetone d_6): τ 4·45 (q, H_2), τ 3·04 (d, $J = 8$ c/s) and τ 2·53 (d, $J = 8$ c/s) ($H_2{}'$, $H_6{}'$, $H_3{}'$ and $H_5{}'$); IR (KBr): 3145, 1672, 1608, 1582, 1522, 1227 and 828 cm^{-1}. An authentic sample of (±) 4′-hydroxyflavanone prepared by the method of Geissman and Clinton[5] agreed in IR, UV and NMR spectra with the experimental sample.

Fraction E—(−)4′-hydroxyflavan-4α-ol (VII). Fraction E yielded a colourless solid, which on crystallization from EtOAc-pet. ether gave colourless crystalline needles (3 mg) m.p. 217–218°, $[\alpha]_D^{25}$ − 71·0 (c, 1·12, acetone). Found: C, 74·5; H, 6·0. Calc. for $C_{15}H_{14}O_3$: C, 74·4; H, 5·8%). It did not answer ferric reaction. It dissolved readily in NaOH aq. With conc H_2SO_4 and HCl, VII gave deep purple colour; $\lambda_{max}^{CH_3OH}$ 225, 275 and 281 sh. nm (log ε, 4·25, 3·52 and 3·47); IR 3472, 1613, 1587, 1522, 1242, 1208, 831 and 756 cm^{-1}; NMR data, $(CD_3)_2CO$: Signals (m) between τ 2·6–3·3 (aromatic protons of rings A and B); two pairs of doublets at τ 2·65 and 3·12 ($J = 9$ c/s, 2′, 6′, 3′ and 5′ protons), τ 4·77 (q, $J_{2,3a} + J_{2,3e} = 14$ c/s, H_2) and τ 5·21 (t, $J_{4,3a} + J_{4,3e} = 6$ c/s, H_4).

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