On the Stereochemistry of the Conversion of Tyramine and Hordenine into Tyrosol by *Willia anomala* Hansen

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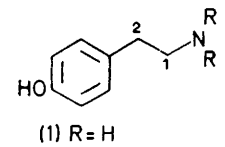
Summary Shaken cultures of *Willia anomala* Hansen convert tyramine (**1**) and hordenine (**2**) into tyrosol (**3**) through a process involving non-stereospecific hydrogen removal from the position α to the nitrogen, followed by hydrogen addition, which, in the case of tyramine (**1**), proceeds with the introduction of a *pro-R* hydrogen atom.

WE report on the stereochemistry of the removal of the hydrogen atom α to the nitrogen and of hydrogen addition, which occur during the conversion of tyramine (**1**) and hordenine (**2**) into tyrosol (**3**) by shaken cultures of *Willia anomala* Hansen.[1] This study was undertaken during a study of the biosynthesis of the *Amaryllidaceae* alkaloids where the stereochemistry of the tritium atom α to the tertiary nitrogen of hordenine (**2**) was required, because we expected that the conversion of the two amines (**1**) and (**2**) might involve stereospecific hydrogen removal from the methylene α to the nitrogen, followed by hydrogen addition.

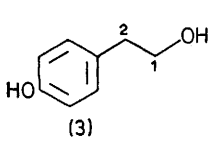
The propionic acid (**4**)[2] (98% ^2H by mass spectrometry) was converted into the methyl ester (**5**), which, upon addition of an excess of PhMgBr in benzene, followed by acid treatment, gave the olefin (**6**). The latter was ozonised to give, after oxidative work up, the acid (**7**). This was converted into the chloride (**8**) ($SOCl_2$ in boiling benzene) and the latter was transformed into the amide (**9**) (Schotten-Baumann). There was no deuterium loss. Reduction of the amide (**9**) with deuteriated diborane led to (**10**), which, on HBr hydrolysis, gave (2*S*) [1-$^2H_2$; 2-$^2H_1$]tyramine (**11**), containing *ca.* 95% $^2H_3$ in 50% overall yield from (**4**). Similarly, starting from (3*S*) [3-$^2H_1$]3-(4-methoxyphenyl)-propionic acid the (2*R*)-trideuterio-amine (**19**) was obtained.

Shaken cultures of *Willia anomala* Hansen converted the

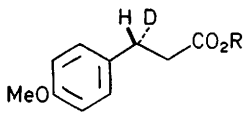
two amines (**11**) and (**19**) into [1,2-$^2H_2$] tyrosols in nearly quantitative yields within 4 days. From (**11**) the *erythro* form, corresponding to the (1*S*,2*S*)-isomer (**12**), was obtained, whereas from (**19**) the *threo* (1*S*,2*R*)-isomer (**17**) was obtained identified by comparison of the n.m.r. spectra of their diacetates with those of authentic samples of *erythro*- and *threo*-diacetyl [1,2-$^2H_2$]tyrosols prepared as follows.



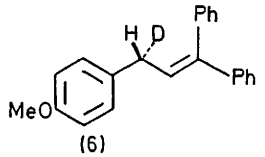
(1) R = H
(2) R = Me



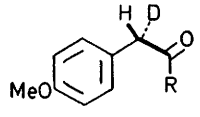
(3)



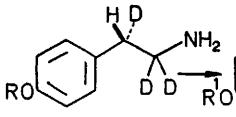
(4) R = H
(5) R = Me



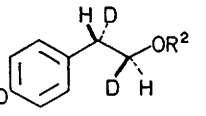
(6)



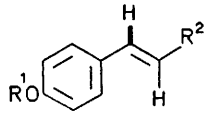
(7) R = OH
(8) R = Cl
(9) R = NH_2



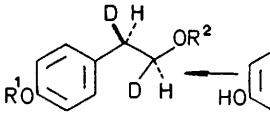
(10) R = Me
(11) R = H



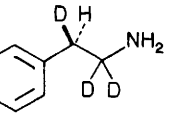
(12) $R^1 = R^2 = H$
(13) $R^1 = CH_2Ph; R^2 = H$
(14) $R^1 = CH_2Ph; R^2 = Ts$



(15) $R^1 = CH_2Ph; R^2 = Br$
(16) $R^1 = CH_2Ph; R^2 = {}^2H$



(17) $R^1 = R^2 = H$
(18) $R = CH_2Ph; R^2 = COPh$



(19)

4-Benzyloxybenzaldehyde was condensed with malonic acid to give *E*-4-benzyloxycinnamic acid, which, upon bromination in CCl_4, gave the α,β-dibromoderivative. This, in turn, upon treatment with $NaHCO_3$ in MeCOEt,[3] led to the *E*-4-benzyloxybromostyrene (**15**) which was converted into 4-benzyloxydeuteriostyrene (96% $^2H_1$), containing at least 90% of the *E*-isomer (**16**).[4] The latter was transformed, upon addition of $B_2{}^2H_6$, followed by oxidative work up,[5] into *erythro*-[1,2-$^2H_2$]4-*O*-benzyltyrosol (**12**). The *p*-toluenesulphonylderivative (**14**), obtained from (**13**) with TsCl in cold pyridine, upon benzoate displacement,[6] was converted into the *threo*-derivative (**18**). The diacetates obtained from (**13**) and (**18**), respectively, through a series of reactions not affecting the diastereoisomeric composition, presented n.m.r. spectra (100 MHz, $CHCl_3$, Me_4Si, with deuterium decoupling) showing, in the aliphatic region, signals at 5·81 and 7·15 (1H each, d) J_{AB} 7·4 and 6·7 Hz, respectively, coincident with those of the 'natural' diacetyldideuterio tyrosols. It follows that the conversion of tyramine (**1**) into tyrosol (**3**) involves the removal of a hydrogen atom from C-1, followed by addition of a *pro-R* hydrogen.

Confirmation in favour of the stereospecificity of the hydrogen addition arose from the following evidence. [1-$^2H_2$]tyramine (**1**) was converted into monodeuteriotyrosol (**3**) by the growing yeast. The [1-$^2H_1$]tyrosol (**3**) was converted into the camphanic ester and its n.m.r. spectrum was recorded in the presence of *ca.* 40% molar excess of $Eu(dpm)_3$. Comparison of this spectrum with that obtained with the undeuteriated material under similar conditions showed the disappearance of the lowest field group of signals of the two corresponding to the methylene protons at C-1 of tyrosol (**3**). This result confirms both the optical purity of the deuterio tyrosol and the efficiency of Gerlach's method.[7]

With the optically active [1-$^2H_1$]tyrosol (**3**) we could synthetise the optically active isomeric tyramines and hordenines, though known procedures,[8] to use later in feeding experiments to determine the stereochemistry of the hydrogen removal. However, repetition of the biological conversion starting from [1-^{3}H; 1-^{14}C]tyramine (**1**) led to tyrosol (**3**) with *ca.* 84% tritium retention. Furthermore, the [1-^{3}H; 1-^{14}C]tyrosol (**3**) was benzylated at the phenolic hydroxy-group and oxidised to [formyl-^{3}H; ^{14}C]-4-benzyloxyphenylacetaldehyde. The latter was reduced with $LiAl^2H_4$ to [1-^{3}H; 1-^{2}H; 1-^{14}C]*O*-benzyltyrosol (98%) $^2H_1$ and converted into [1-^{3}H; 1-^{2}H; 1-^{14}C]tyramine (**1**). The triply labelled compound was transformed into tyrosol (**3**) with *ca.* 69% tritium and 75% deuterium retentions, respectively.

[1-^{3}H; 1-^{14}C]Hordenine (**2**) was converted into tyrosol (**3**) with *ca.* 96% tritium retention.

The evidence therefore suggests that, within the accuracy of the radioactivity and mass spectrometric measurements, the hydrogen removal from the methylene α to the nitrogen occurring in the biological conversion of tyramine (**1**) and hordenine (**2**) into tyrosol (**3**) is non stereospecific, and proceeds, apparently, with different k_H/k_T values, whereas the hydrogen addition, as determined for tyramine (**1**), involves the introduction at C-1 of a *pro-R* hydrogen.

The first results contrasts the stereospecificity of the oxidation observed for tyramine (**1**) in animal systems.[8]

We thank Prof. Hans Gerlach for a sample of (−)-camphanoyl chloride, Mr. Giulio Zannoni, ICM, Milano, for the n.m.r. spectra with deuterium decoupling, and Mr. Gianni Bertolli for technical assistance.

(*Received*, 20*th July* 1973; *Com.* 1054.)

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