SPECIALIA

On the synthesis of serine and homoserine samples asymmetrically labelled with tritium and deuterium in the hydroxymethylene group

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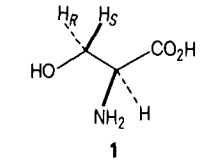
Summary. (1*R*)-[1-^{3}H, ^{2}H$_1$] 3-Phenylpropanol, the key intermediate in the synthesis of (4*R*)-[4-^{3}H, ^{2}H$_1$] D,L-homoserine and of the (4*S*)-isomer, is obtained from (1*S*)-[1-^{2}H$_1$] 3-phenylpropanol and (1*RS*)-[1-^{3}H] ethanol upon incubation with yeast alcohol dehydrogenase and NAD$^+$; under similar conditions 2-phenylethanol undergoes very small exchange with [1-^{2}H$_2$] ethanol.

The mechanism of pyridoxal phosphate (PLP) dependent enzymic reactions of the amino acids serine (**1**) and homoserine (**2**) has been interpreted[1,2] on the basis of the properties of the Schiff base formed between enzyme-bonded PLP and the amino acids on the reaction pathway. Information on the conformation of the enzyme-bonded intermediates dictated, in turn, from the nature and the geometry of the enzymes active sites, is being currently obtained from the stereochemical analysis of a selected set of enzymic reactions using serine (**1**)[3a–h] and homoserine (**2**)[4] asymmetrically labelled with isotopic hydrogen.

However, investigations on the mechanism of enzymic reactions of the above amino acids involving methylene → methyl conversion require that samples of amino acid asymmetrically labelled with deuterium and tritium in the hydroxymethylene group are available, and a synthesis of the enantiomeric forms of L-serine (**1**) stereospecifically labelled with deuterium and tritium in position 3, based on enzymic exchange procedures, has been reported[3h].

We outline here the results of experiments designed to obtain the alcohols **3** and **4**, the key intermediates in our syntheses of asymmetrically labelled **1**[3b] and **2**[5], asymmetrically labelled with ^{3}H and ^{2}H at position 1. The general procedure[6] reported for stereospecific labelling of alcohols involving enzymic reduction of the labelled aldehyde, if used for multiple isotopic labelling, seemed here not to be of facile application due to the nature of the aldehydic species involved. We therefore turned our attention to the exchange reaction[7] of a deuterium atom at position 1 of 3-phenylpropanol (**4**) for a *pro-R* hydrogen atom carried on by fermenting baker's yeast. In the expectation that the exchange might be the consequence of the reversility of the NAD-dependent dehydrogenase reaction, leading eventually to the distribution of the *pro-R* hydrogen species removed from 3-phenylpropanol (**4**) to give the reduced form of the cofactor in the *pro-R* position of all the alcoholic species present in the fermentation mixture, and taking part to this type of equilibria, we incubated 3-phenylpropanol (**4**), 3.7 mmoles, with purified yeast alcohol dehydrogenase (EC 1.1.1.1, ex Boehringer), 30 mg, NAD$^+$, 40 mg and 1-monodeuterioethanol, 15.6 mmoles, in 200 ml of phosphate-glycine buffer, pH 9, room temperature, and found in the 3-phenylpropanol isolated after 15, 40 and 70 h the presence of 15%, 25% and 30%, respectively, of d_1 species. The expected deuterium content of **4** for an equilibrium distribution of deuterium in the *pro-R* position of the 2 alcohols is 40%. Complementary results were obtained using [1-^{2}H$_2$] 3-phenylpropanol and un-



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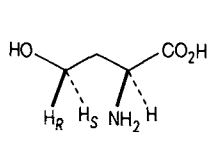

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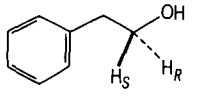

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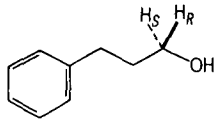

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labelled ethanol, whereas ^{1}H-NMR studies[7,8] on the camphanoyl derivatives confirmed the *pro-R* absolute configuration of the exchanged hydrogen atom.

The above results opened the way to synthetise the required (**1***R*) [1-^{3}H, ^{2}H$_1$] 3-phenylpropanol, using sodium borotritiide as primary ^{3}H source. Thus, incubation of (**1***S*) [1-^{2}H$_1$] 3-phenylpropanol (4, $H_S = {}^2H$), 99% d_1, 10 mmoles, with (**1***RS*) [1-^{3}H] ethanol, 1 mmole, about 100 mCi, obtained upon NaB^{3}H$_4$ reduction of acetaldehyde, gave (**1***R*) [1-^{3}H, ^{2}H$_1$] 3-phenylpropanol (4, $H_R = {}^3H$, $H_S = {}^2H$), 4.5 mCi/mmole, 99 d_1, in nearly quantitative chemical and overall acceptable radiochemical yields. Conversion to (**4***R*) [4-^{3}H, ^{2}H$_1$] D,L-homoserine and to the (**4***S*)-isomer was carried on as reported[5] and proceeded without tritium loss.

Repetition of this type of experiments, using [1-^{2}H$_2$] 2-phenylethanol or unlabelled **3** and [1-^{2}H$_2$] ethanol, indicated a negligeable isotopic exchange, thus suggesting that at present the labelling procedure reported is unsuitable for the synthesis of ^{3}H, ^{2}H-asymmetrically labelled serine if a high ^{3}H-specific activity is required. However, since (**1***S*) [1-^{2}H$_1$] 2-phenylethanol (3, $H_S = {}^2H$) is obtained in growing cultures of *Willia anomala* Hansen from [1-^{2}H$_2$] 2-phenyl ethylamine through a process which we now know from experiments with asymmetrically labelled amine to involve removal of the *pro-R* hydrogen atom from the position α to the nitrogen atom, followed by reduction of the intermediate phenylacetaldehyde, experiments designed to introduce tritium in the C_6–C_2 alcohol in the reduction step are in progress.

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A p-menthane derivative isolated from culture filtrates of *Fusicoccum amygdali*, Del.

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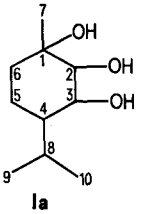
Summary. From culture filtrates of *Fusicoccum amygdali*, Del., a new compound, whose structure corresponds to 1,2,3-trihydroxy-p-menthane, has been isolated. Its discovery is of some interest since, to our knowledge, it is the first time that a monoterpenoid is isolated from a microorganism.

Submerged cultures of *Fusicoccum amygdali*, Del. have been known to produce fusicoccin[3], a highly phytotoxic diterpene glucoside, along with a number of closely related co-metabolites[4–11], each with a characteristic diterpene aglycone. An extremely careful separation of the components of culture filtrates allowed us to isolate an entirely different, novel compound possessing the para-menthane skeleton. Spectroscopic and chemical evidence reported below showed it to be of structure **I**. To our knowledge, this is the 1st case that a monoterpenoid had been isolated from cultures of a microorganism. Further studies, however, are required to ascertain whether or not **I** represents a true metabolite of *Fusicoccum amygdali*, Del.



Ia

Compound **I** was obtained in low (0.5%) yields by extensive and repeated chromatographic fractionation of the residue left in ethyl acetate after crystallization of the major metabolite. Several crystallizations from benzene of the newly isolated product gave white prisms m.p. 86–87 °C. Its molecular formula $C_{10}H_{20}O_3$ resulted from elemental analysis. The IR-spectrum (CCl_4) showed sharp bands at 3625 and 3575 cm^{-1} indicating the presence of at least 2 alcoholic functions. The ^{1}H-NMR-spectrum (in a 3:1 mixture of $CDCl_3$ and DMSO-d_6, at 100 MHz) revealed the presence of 2 secondary and 1 tertiary hydroxyl groups. It also showed that the molecule has 1 secondary isopropyl and 1 tertiary methyl group and, furthermore, has a fully saturated hydrocarbon backbone.

The above findings may readily be accomodated in the monoterpenoid structure **Ia** with the following assigned ^{1}H-NMR-parameters: $\delta_{CDCl_3 + DMSO} = 0.98$ (6H, d, 6.7 Hz 9 $-CH_3$, 10 $-CH_3$); 1.32 (3H, s, 7 $-CH_3$); 1.66 (1H, m, C_8-H); 3.13 (1H, d, 4 Hz, exchangeable, C_3 –OH); 3.33 (1H, s, exchangeable, C_1 –OH); 3.45 (1H, dd, 3 Hz, 4.8 Hz, C_2 –H); 3.79 (1H, d, 3 Hz, exchangeable, C_2OH); 4.03 (1H, ddd, 2 Hz, 4 Hz, 4.8 Hz, C_3 –H) ppm. The stereochemistry of the molecule was derived on the basis of the following arguments.

The magnitude of the vicinal coupling constant J_{34} (2 Hz) suggests that the substituents at C_3 and C_4 are cis oriented. In fact, assuming that conformational free energies of substituents in **Ia** are nearly additive (2.15, 1.7 and 0.7 kcal·mole^{-1} for iPr, CH_3 and OH groups, respectively[12]), trans diaxial arrangement of C_3 and C_4 substituents must be greatly destabilized, whereas their diequatorial orientation is expected to result in a much higher value of J_{34} (approx. 8–10). Among the possible 2 cis conformers, the 1 with axial iPr and equatorial C_3 –OH would again give rise to a greater J_{34} (approximately 5 Hz) and, also the

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