Experimental Section

3g (representative procedure): A mixture of (*S*)-*tert*-leucine dimethylamide (**2g**; 696 mg, 4.40 mmol), oxoester **1a** (749 mg, 4.40 mmol), and molecular sieves (4 Å, 2.5 g) in toluene (6 mL) under nitrogen was treated with a catalytic amount of concentrated HCl. After stirring for 14 h at 60 °C, the reaction mixture was filtered and the residue washed with CH_2Cl_2. All volatile materials were removed in vacuo and the residue was chromatographed on Al_2O_3 90 (Activity II–III; eluent = methyl *tert*-butylether/petroleum ether 3/1; $R_f = 0.45$) to yield **3g** as a colorless solid (1.17 g, 3.78 mmol, 86 %). m.p. 104 °C; $[\alpha]_D^{20} = +194$ ($c = 5.8$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.05$ (s, 9H), 1.25 (t, $J = 7.1$ Hz, 3H), 1.47–1.58 (m, 2H), 1.58–1.67 (m, 2H), 1.99–2.09 (m, 1H), 2.22–2.34 (m, 3H), 2.97 (s, 3H), 3.11 (s, 3H), 4.09–4.20 (m, 2H), 4.24 (d, $J = 9.9$ Hz, 1H), 9.45 (d, $J = 9.8$ Hz, 1H); ^{13}C{^{1}H} NMR (50 MHz, $CDCl_3$): $\delta = 14.61$ (CH_3), 22.43 (CH_2), 22.55 (CH_2), 23.97 (CH_2), 26.66 (CH_3), 26.76 (CH_2), 35.74 (CH_3), 35.96 (C), 37.90 (CH_3), 57.75 (CH), 58.72 (CH_2), 91.27 (C), 156.47 (C), 170.41 (C=O), 171.67 (C=O); HR MS (EI, 70 eV): calcd: 310.2256, found: 310.2249; elemental analysis (%): calcd for $C_{17}H_{30}N_2O_3$ (310.44): C 65.77, H 9.74, N 9.02; found: C 65.94, H 9.76, N 9.16.

Copper(II)-catalyzed Michael reaction of **3a** (representative procedure): Enaminoester **3a** (0.216 mmol, 70.1 mg) and $Cu(OAc)_2 \cdot H_2O$ (**5a**; 0.0054 mmol, 1.1 mg) were stirred in acetone (1 mL) at 23 °C for 1 h. MVK (**4**; 0.43 mmol, 30 mg) was added and the mixture was stirred for additional 12–14 h at 23 °C. All volatile materials were removed in vacuo and the residue was treated with 2 N HCl. The mixture was stirred vigorously for 4–5 h and subsequently extracted with methyl *tert*-butylether.[16] After washing (saturated aqueous $NaHCO_3$) und drying ($MgSO_4$) of the combined extracts, the solvent was evaporated and the residue was chromatographed on SiO_2 (methyl *tert*-butylether/petroleum ether 1/2, $R_f = 0.19$). Compound **6a** (0.186 mmol, 44.6 mg, 86 %) was obtained as a colorless oil. The *ee* value of 98 % was determined by GC with a chiral column after derivatization to compound **7a**.[15]

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[12] Syntheses of auxiliaries **2a**–**j** were accomplished in three steps from the appropriate α-amino acids: a) Boc_2O, DMAP (3 mol %), H_2O/MeOH 1/1, 70–80 %; b) DCC, HNR'_2, CH_2Cl_2, 65–85 %; c) CF_3CO_2H, CH_2Cl_2, 90–99 %. Boc = *tert*-butoxycarbonyl, DMAP = 4-dimethylaminopyridine, DCC = dicyclohexylcarbodiimide.

[13] Syntheses of auxiliaries **2m**–**p** were accomplished in four steps from the appropriate amino alcohols: a) Boc_2O, CH_2Cl_2, quantitative; b) TosCl, pyridine, 65–90 %; c) for **2m**, **n**: NaSEt, DMF, 90–95 %; for **2o**, **p**: $HNMe_2$, pyridine, 75–95 %; d) CF_3CO_2H, CH_2Cl_2, 90–99 %. Tos = *p*-$H_3CC_6H_4SO_2$.

[14] Applied metal salts: $Cu(OAc)_2 \cdot H_2O$ (**5a**), $Cu(OTf)_2$ (**5b**), $FeCl_3$ (**5c**), $FeCl_3 \cdot 6\,H_2O$ (**5d**), $NiCl_2$ (**5e**), $Ni(OAc)_2 \cdot 4H_2O$ (**5f**), $SnCl_2$ (**5g**), AgOAc (**5h**), $CoCl_2 \cdot 6H_2O$ (**5i**), $CrCl_3 \cdot 6H_2O$ (**5j**), $MgBr_2$ (**5k**), $Pb(OAc)_2 \cdot 3H_2O$ (**5l**), $ZnBr_2$ (**5m**), $ZnCl_2$ (**5n**). Tf = F_3CSO_2.

[15] Details about GC analysis and derivatization procedures have recently been reported by us.[8]

[16] Auxiliary **2** can be almost quantitatively recovered from the aqueous layer after basic workup.

Detection of a 2,3-Aminomutase in the Mushroom *Cortinarius violaceus***

Peter Spiteller, Matthias Rüth, Franz von Nussbaum, and Wolfgang Steglich*

Recently, we discovered the new natural β-amino acid (*R*)-3,4-dihydroxy-β-phenylalanine ((*R*)-β-dopa, (*R*)-**3**) in the mushroom *Cortinarius violaceus*.[1] (*R*)-**3** is present in the mushroom as the iron(III)–catechol complex, which gives the fruit bodies their blue-purple color.

In this communication we report on the biosynthesis of (*R*)-β-dopa. For this purpose, suitable precursors were applied to young fruit bodies of *C. violaceus*, which were then harvested 5 to 7 days later. All mushrooms showed normal growth and became double their original size. After extraction of the fruit bodies with methanol, the amino acids were isolated by ion exchange chromatography and further investigated by GC/MS as their trimethylsilyl derivatives. The incorporation of ^{13}C-labeled precursors was determined by NMR spectroscopy.

After feeding with *rac*-3-fluorotyrosine, we were able to detect the formation of 5-fluoro-β-dopa as well as traces of 3-fluoro-β-tyrosine (Table 1, entry 1).[2] This proves that tyrosine (**1**) is the biosynthetic precursor of β-dopa (**3**). This result is confirmed by the successful conversion of *rac*-[3′-

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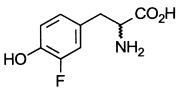
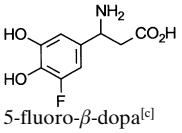
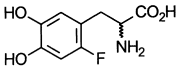
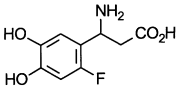
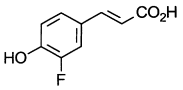
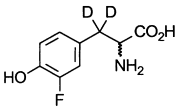
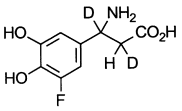
Table 1. Incorporation results after feeding labeled precursors to *C. violaceus*.

entry	precursor[a]	expected product	incorporation [%][b] (method)
1			3.5 (GC/MS)
	3-fluorotyrosine	5-fluoro-β-dopa[c]	
2	[3′-^{13}C]tyrosine	[3′-^{13}C]β-dopa	1.4 (^{13}C NMR)
3			0.0 (GC/MS)
	6-fluorodopa	6-fluoro-β-dopa	
4	[3′-^{13}C]dopa	[3′-^{13}C]β-dopa	0.0 (^{13}C NMR)
5	3-fluoro-β-tyrosine	5-fluoro-β-dopa	1.2 (GC/MS)
6		5-fluoro-β-dopa	0.0 (GC/MS)
	3-fluoro-4-hydroxycinnamic acid		
7	3-fluoro[^{15}N]tyrosine	5-fluoro[^{15}N]β-dopa	3.7 (GC/MS)
8			3.2 (GC/MS)
	3-fluoro[3′,3′-^{2}H$_2$]tyrosine	5-fluoro[2′,3′-^{2}H$_2$]β-dopa	
9	(*S*)-3-fluorotyrosine	(*R*)-3-fluoro-β-tyrosine[d]	1.4 (GC/MS)
10	3-fluoro-β-tyrosine	(*R*)-5-fluoro-β-dopa	1.5 (GC/MS)

[a] Racemic unless otherwise indicated. [b] In the GC/MS experiments the ratio of the base peaks of the labeled and unlabeled compounds is given in percent. In the ^{13}C NMR experiments the incorporation rate is defined as percentage of atom excess. [c] As well as 3-fluoro-β-tyrosine. [d] As well as (*R*)-5-fluoro-β-dopa.

^{13}C]tyrosine into β-dopa (Table 1, entry 2). In contrast, neither feeding with *rac*-6-fluoro-dopa nor with *rac*-[3′-^{13}C]dopa gave any labeled β-dopa (Table 1, entries 3 and 4). Therefore, we conclude that the biosynthesis of β-dopa (**3**) proceeds through β-tyrosine (**2**) which is then hydroxylated. This assumption was confirmed by a successful feeding experiment with *rac*-3-fluoro-β-tyrosine (Table 1, entry 5). In addition, traces of β-tyrosine were detected in the fruit bodies by GC/MS, after suitable derivatization.

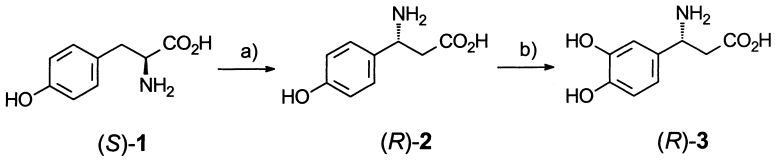
(*S*)-β-Tyrosine (**2**) is a constituent of the edein complex of antibiotics formed by *Bacillus brevis*. In this bacterium **2** is produced from tyrosine through 4-hydroxycinnamic acid by ammonia elimination and subsequent lyase-catalyzed reintroduction of the amino group in the β-position.[3] In contrast, we were unable to recognize any incorporation of 3-fluoro-4-hydroxycinnamic acid into β-dopa with *C. violaceus* (Table 1, entry 6). In order to exclude the elimination–addition sequence unambiguously, *C. violaceus* was fed with 3-fluoro[^{15}N]tyrosine. The successful incorporation of this compound into (*R*)-β-dopa verifies that the amino shift proceeds with retention of the nitrogen (Table 1, entry 7). Therefore, the biosynthesis of (*R*)-β-dopa in *C. violaceus* follows the sequence depicted in Scheme 1.

The tyrosine-2,3-aminomutase from *C. violaceus* is comparable to the enzyme discovered by Floss[4] in extracts of *Taxus brevifolia*. This aminomutase catalyses the transformation of (*S*)-α-phenylalanine into (*R*)-β-phenylalanine during the side-chain construction of paclitaxel.

In aminomutase-catalyzed reactions the pro-3*S* hydrogen is usually substituted by the migrating amino group, under retention of configuration at C-3, and concomitant intramolecular transfer of a hydrogen atom from C-3 to C-2.[5–8] This reaction was shown to occur also in *C. violaceus*: after feeding with 3-fluoro[3′,3′-^{2}H$_2$]tyrosine, the formation of 5-fluoro[2′,3′-^{2}H$_2$]β-dopa could be recognized by mass spectrometry (Table 1, entry 8).

In order to investigate the specificity of the enzyme, (*S*)-3-fluorotyrosine, as well as the racemic mixture, was applied to *C. violaceus*. The stereochemistry of the resulting 3-fluoro-β-tyrosine was determined by GC after derivatization with Mosher's acid chloride.[9] Since in both experiments only the (*R*)-enantiomer of 3-fluoro-β-tyrosine was detected, the tyrosine-2,3-aminomutase transforms exclusively (*S*)-3-fluorotyrosine (Table 1, entry 9). Likewise, the hydroxylation of 3-fluoro-β-tyrosine to 5-fluoro-β-dopa is strictly stereospecific (Table 1, entry 10).

The tyrosine-2,3-aminomutase from *C. violaceus* is the first aminomutase detected in a fungus. The transfer of the amino group in all known enzymes of this type occurs via radical



Scheme 1. Biosynthesis of (*R*)-β-dopa in *C. violaceus*: a) tyrosine-2,3-aminomutase; b) monooxygenase.

intermediates,[10] whereby either adenosylcobalamin[11] or S-adenosylmethionine (SAM)[6, 11, 12] serve as cofactors. Since corrinoids are not produced by fungi,[13] adenosylcobalamin is excluded as a cofactor in our case. At present it is not possible, however, to decide whether the tyrosine-2,3-aminomutase of *C. violaceus* is a SAM-dependent enzyme or an aminomutase of a new type.[4]

Experimental Section

The labeled α-amino acids were synthesized according to the phthalimidomalonic ester method; 3-fluoro-4-hydroxycinnamic acid was prepared by Knoevenagel condensation. 3-Fluoro-β-tyrosine was synthesized according to the method of Rodionov and Postovskaja,[14] commencing with 3-fluoro-4-methoxybenzaldehyde, diethyl malonate, and ammonium acetate.

Feeding experiments: Each of the labeled compounds (50 mg) was dissolved in water (0.2 mL) and injected into between one and five fruit bodies of *C. violaceus* in their natural environment.[15] The mushrooms were harvested after 5–7 d and immediately frozen. The finely crushed fruit bodies (50 g) were thoroughly extracted with a mixture of methanol (250 mL) and 2 N HCl (2 mL) at room temperature. The extract was filtered

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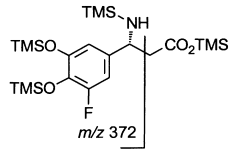
and the solvent completely removed in vacuo. The residue was dissolved in water (5 mL) and subjected to ion-exchange chromatography (Dowex 50W X8, eluent 4 N HCl) to remove the nonbasic components. The eluent was lyophilized and the residue dissolved in methanol (2 mL). In order to remove Fe^{III} ions, NaSCN (50 mg) was added. Chromatography on Sephadex LH-20 yielded 45 mg of the amino acids (0.09 % of fresh weight).

Determination of the incorporation of labeled precursors: The isolated amino acids were silylated with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and subsequently analyzed by GC/MS. The incorporation of single fluoro-labeled precursors was determined by measuring the ion current of the base peak derived from **4** (*m*/*z* 372). In the case of double-labeled precursors the ion *m*/*z* 373 was used. If ^{13}C-labeled precursors were used the incorporation rates were determined by ^{13}C NMR measurements.



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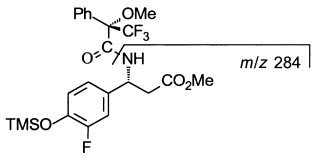
In order to determine the configuration of the derived 3-fluoro-β-tyrosine, the amino acid (0.5 mg) was esterified with methanol (0.5 mL) and Me_3SiCl (100 μL). After removal of the solvent, the residue was dissolved in pyridine (100 μL). (*R*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid chloride (2 μL) and a catalytic amount of 4-dimethylaminopyridine (DMAP) were added, and the mixture was kept at room temperature for 12 h. The solvent was then removed and the residue treated with MSTFA (40 μL). The amino acid derivative **5** was identified by GC/MS monitoring of the ion *m*/*z* 284. Temperature program: 2 min isotherm at 50 °C, then 10 K min^{-1} to 300 °C, followed by 15 min isotherm at 300 °C. Column: fused-silica capillary column (DB-5 ms, J&W, 30 m × 0.25 mm × 0.25 μm). The Kováts indices were determined by coinjection with a mixture of straight chain saturated hydrocarbons (C_{10}–C_{36}). (2′*S*,3*R*)-**5**: GC: $R_i = 2622$; (2′*S*,3*S*)-**5:** GC: $R_i = 2649$.



(2′*S*,3*R*)-**5**

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Demonstration of Chiral Enantiomerization in a Four-Atom Molecule**

Paul von Ragué Schleyer* and Michael Mauksch

In 1997, we presented the first example of the enantiomerization of a chiral five-atom molecule, *cyclo*-SeSSOS, via a completely asymmetric reaction path in a single-step process.[1] Is it possible for a chiral molecule with only four atoms to enantiomerize via a chiral pathway? The answer is "no" if all four atoms are different. Oddly, the answer may be "yes" if two of the four atoms are the same.

In 1992 and 1993, Mislow et al. presented an elegant proof that fully chiral paths might be involved in the interconversion of mirror images of asymmetric (geometrically distorted) tetrahedra, *which are not fully labeled*.[2, 3] In contrast, Mislow demonstrated in 1995 that this is an exception: No path for interconverting mirror images can preserve chirality for fully labeled, three-dimensional, four-vertex simplexes.[4] These must pass through a two-dimensional boundary in order to reach the enantiomorphic forms.[5–7] Later in 1995, Mezey explained this conundrum, "Mislow's Labeling Paradox",[8, 9] by showing, in an abstract form (Figure 1), that chiral four-vertex simplexes, in which at least two identically labeled vertices can switch roles (for example, as a permutation of sites), may be transformed to their enantiomorphs on a fully asymmetric reaction path.[10] In tetraatomic molecules, such a "permutation of sites", which leads to an enantiomer, can occur only if two atoms of the same chemical element and isotope have distinguishable sites and exchange their chemical and spatial environments.

Weinberg and Mislow's 1996 dimensional analysis[11] showed that all submaximally labeled chiral simplexes are chirally connected (for example, asymmetric tetrahedrons with at least two identical atoms can be converted into their

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