112. Novel Synthesis of Agaritine, a 4-Hydrazinobenzyl-Alcohol Derivative Occurring in Agaricaceae

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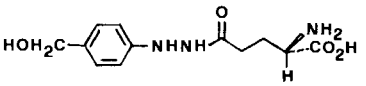
The 4-hydrazinobenzyl alcohol (**3**) was prepared (58%) by diisobutylaluminiumhydride reduction of methyl 4-hydrazinobenzoate (**4**), whereas $LiAlH_4$ or $LiBH_4$ reduction of **4** proceeded further to yield (*via* intermediate **3**) (4-tolyl)hydrazine (**5**). The alcohol **3** was stable under O_2-free conditions and exhibited no tendency to eliminate H_2O, neither thermally nor with H^+ catalysis. Oxidation of **3** with SeO_2 yielded 4-(hydroxymethyl)benzenediazonium ion (**8**), identified by its azo coupling product **9** with 2-naphthol. Condensation of **3** with 1-benzyl 5-hydrogen *N*-(benzyloxycarbonyl)-L-glutamate (**10**) in presence of dicyclohexylcarbodiimide afforded 81% of N^2-(benzyloxycarbonyl)-L-glutamic acid 1-(benzyl-ester) 5-{2-[4-(hydroxymethyl)phenyl]hydrazide} (**11**) which upon controlled hydrogenolysis (quinoline-sulfur-poisoned Pd/C catalyst) gave 82% of L-glutamic acid 5-{2-[4-(hydroxymethyl)phenyl]hydrazide} (**1**), *i.e.* agaritine, a metabolite of *Agaricus bisporus*. Without poisoning of the catalyst, hydrogenolysis of (**11**) yielded L-glutamic acid 5-[2-(4-tolyl)hydrazide] (**12**).

Introduction. – Agaritine (**1**), one of the relatively rare natural products containing a N–N bond in their molecular structure [1], has been found in several species of the genus *Agaricus* [2] [3]. The notable occurrence [3] [4] in the commercial mushroom (*Agaricus bisporus*) is responsible for the interest of natural-product chemists [5] in its biosynthesis, of biochemists [2] [6] in its metabolic transformations, and of toxicologists [7] in its potential toxicity and cancerogenicity as a phenylhydrazine derivative. Further studies in these fields depend on the ready availability of agaritine (**1**), particularly, perhaps, as a specifically labeled isotopomer. For this, chemical synthesis appeared to be better suited at present than biosynthesis and extraction from the mushroom.

Two syntheses of **1** are known, both starting from 4-hydrazinobenzoic acid (**2**). The first method [8] yields only 1% of **1** (based on **2**) and the second [9], while claiming a 23% yield, could not be reproduced by us and others[2]). We, therefore, looked for another synthesis of **1**. Our scheme includes as its key step the condensation of 4-hydrazinobenzyl alcohol (**3**) with the 5-carboxy group of properly protected and activated L-glutamic acid.



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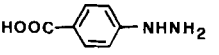

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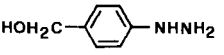

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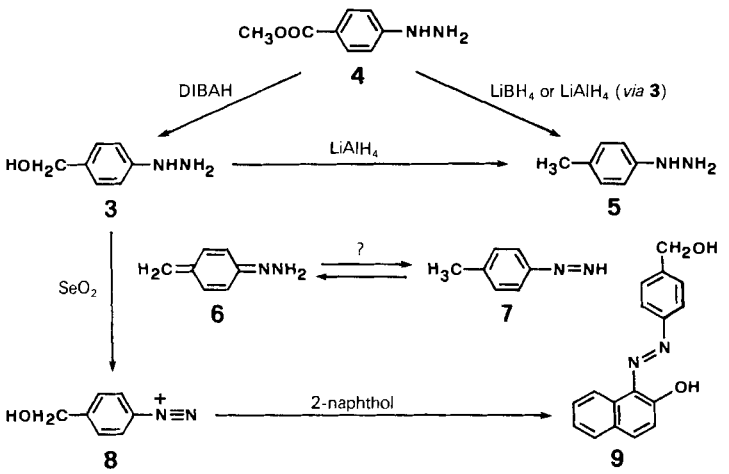
[1]) Postdoctoral fellow on leave from West Bengal (State) Education Service, Calcutta, India.

[2]) Dr. *J. Lüthy*, Institute of Toxicology of the University of Zürich and of the ETH Zürich, informed us about fruitless attempts on the synthesis of **1** according to [9]: according to [3], the method of [9] had to be modified substantially, leading finally to **1** in only 95% purity.

This obvious approach has already been used in the first synthesis of **1** [8], however, with *in-situ* preparation of **3**, only, and using the not too easily available *N*-(benzyloxycarbonyl)-L-glutamic acid 5-azide as the coupling partner. No attempts had been made to isolate **3** because of an assumed [8] facile elimination of H_2O to yield the hydrazone **6** (see below, *Scheme 1*). A low stability of **3** due to this latter reaction was claimed to be responsible for the low yield of **1** in [8].

Scheme 1



Here, we report on the synthesis of **1** in 33% yield (based on **2**) *via* the isolated 4-hydrazinobenzyl alcohol (**3**) by its condensation with 1-benzyl 5-hydrogen *N*-(benzyloxycarbonyl)-L-glutamate (**10**). We also describe some properties of **3** which revealed its resistance against elimination of H_2O, thus correcting literature claims [8] [9].

Synthesis of 4-Hydrazinobenzyl Alcohol (3). – Hydride reduction of an ester of 4-hydrazinobenzoic acid (**2**) is an obvious route for the preparation of **3** [10]. However, since the product is a benzyl alcohol which might tend to be overreduced to the corresponding tolyl derivative [11] $LiAlH_4$ is unsuitable[3]). Even the milder $LiBH_4$ [12] in THF reduced methyl 4-hydrazinobenzoate (**4**) to **3** and (4-tolyl)hydrazine (**5**) at a comparable rate (TLC evidence; see *Scheme 1*). The overreduction was avoided with diisobutylaluminium hydride (DIBAH) in toluene at −70°: it reduced **4** to 58% of **3** under O_2-free conditions (see *Scheme 1*).

The structure of **3** is supported by its spectroscopic properties (see *Exper. Part*). The UV spectrum (278 (sh, ε = 2500), 238 (11 650)) is rather similar to those of agaritine (**1**) [8] and of (4-tolyl)hydrazine (**5**) [13], and the MS (70 eV) shows the signal of $M^{+\cdot}$ as base peak at *m/z* 138, followed by only few intensive peaks, namely at *m/z* 121 (81%, loss of OH), 92 (36%, loss of H_2O and N_2), and 77 (45%, $C_6H_5^+$).

Chemical reactivity also supports the structural assignment of **3**. Oxidation of **3** with SeO_2 [8] and treating the reaction mixture with 2-naphthol gave the azo-coupling product

[3]) *Kelly et al.* [8] used $LiAlH_4$ for their *in-situ* generation of **3** from **4**, but they used somewhat special conditions; their low yield of the final product **1** might be due to overreduction of **4** to (4-tolyl)hydrazine (**5**).

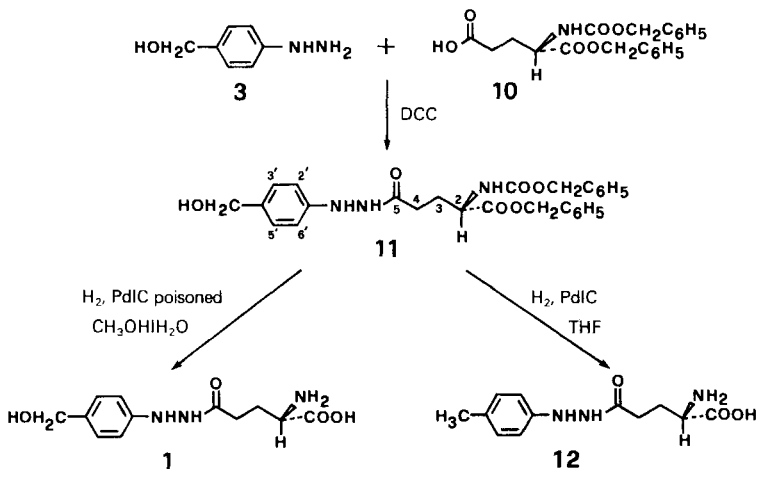
9, identical with the product of coupling 4-(hydroxymethyl)benzenediazonium ion (**8**) with the same naphthol [14] (see *Scheme 1*). Reduction of **3** with $LiAlH_4$ or $LiBH_4$ (see above) led to (4-tolyl)hydrazine (**5**; see *Scheme 1*). The coupling of **3** with the 5-carboxy group of the 1-carboxy-protected *N*-(benzyloxycarbonyl)-L-glutamic-acid derivative **10** afforded, after deprotection, agaritine (**1**; see below, *Scheme 2*).

The hydrazine **3** showed a remarkable thermal stability. Actually, the most efficient way of its purification was bulb-to-bulb distillation at $140°/2 \cdot 10^{-4}$ Torr. In contrast to previous claims [8] [9], **3** was also stable in aqueous solution, even at low pH (HCl/KCl buffer, pH 2.0) for at least 4 d, as shown by monitoring with UV. Since the UV spectra of the dehydration product **6** or its tautomer, the (4-tolyl)diazene (**7**; see *Scheme 1*), should differ from that of protonated **3** (as expected for **7** [13]), the unchanged UV spectrum of **3** at pH 2 means that no acid-catalyzed elimination of H_2O had occurred (this does not exclude **6** as a minor component in an equilibrium with **3**).

The only common reagent harmful to **3** under ordinary conditions is O_2; the primary arylhydrazino [15] and the benzyl-alcohol group [16] are susceptible to oxidation. When keeping solid **3** in contact with air, it rapidly turns yellow to orange. The TLC of this colored material shows the presence of at least 5 decomposition products: none of them has been isolated.

Synthesis of Agaritine (1). – Condensation of **3** with the 1-benzyl 5-hydrogen *N*-(benzyloxycarbonyl)-L-glutamate (**10**)[4]) in presence of dicyclohexylcarbodiimide (DCC) took place exclusively at the *N'*-position of **3** yielding 81 % of N^2-(benzyloxycarbonyl)-L-glutamic acid 1-(benzyl ester) 5-{2-[4-(hydroxymethyl)phenyl]hydrazide} (**11**; see *Scheme 2*). No undesired competition products such as the ones from the acylation of the OH group or of the N-atom next to the phenyl ring in **3** were observed (*cf.* [9]).

Scheme 2



[4]) The acid **10** has been described [17] only as its dicyclohexylammonium salt. We used the free acid and report some of its physical properties in the *Exper. Part.*

The product **11** is already known as an intermediate of the second synthesis [9] of agaritine (**1**), although it was not completely characterized by standard spectroscopy (see *Exper. Part*). The ^{1}H-NMR spectrum of **11** in (D_6)DMSO which shows the expected signals for all 29 H-atoms deserves a few remarks. At r.t., 3 H-atoms are rapidly exchangeable with D_2O and 1 H-atom (a pair of signals) exchanges only slowly (several at r.t.). The first are the benzylic OH proton producing a *t* (J = 5.5 Hz) at 4.90 ppm by coupling with the benzylic CH_2 protons (*d* at 4.33 ppm (J = 5.5 Hz), becoming a *s* after D_2O-exchange) and those of 2 NH protons at 9.62 and 7.61 ppm. Since the latter signals both are *d* with the same (vicinal) coupling constant (J = 2.6), they must be assigned to the hydrazino group in **11**, the signal at lower field most likely to NH–C(5) because of its amide-type environment.

The H-atom undergoing slow D_2O exchange produces 2 *d* (J = 8.0) at 7.86 and 7.76 ppm in the ratio of *ca.* 4:1; they are assigned to NH–C(2) of 2 amide rotamers in slow exchange at r.t. due to restricted rotation around the N–C(=O) bond in the carbamoyl moiety (see *Figure*). One of the CH_2 groups of the 2 benzyloxycarbonyl groups also causes two *s* at 5.06 and 5.08 ppm in the ratio of again 4:1, together for 2 H-atoms. This pair is attributed to the N-protecting benzyloxycarbonyl group which is closer to the amide functionality. The other benzyloxycarbonyl group gives rise to only 1 CH_2 *s* at 5.15 ppm. At 40°, as expected for amide rotamers [18], the 2 signal pairs become fused to a 1-H *d* (J = 8.0) at 7.60 ppm (NH–C(2)) and a 2-H *s* at 5.06 ppm (CH_2) by a more rapid exchange.

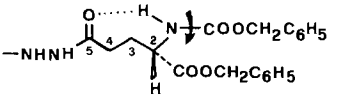


Figure. *Partial structure of* **11** *with H-bonding between* NH–*C(2) and* O=*C(5)*. Restricted rotation around the N–C(=O) bond in the carbamoyl moiety is indicated by an arrow.

The relative slow rate of H/D-exchange of NH–C(2) suggests that this H-atom is engaged in a relatively strong H-bond, perhaps an intramolecular one with O=C(5) as the acceptor (see *Figure*). At 60°, this H-bond becomes more mobile as indicated by the change of the NMR signal for NH–C(2) into a broad *s*.

Removal of the protecting benzyl groups from the 1-carboxy and from the carbamoyl moiety of **11** by catalytic hydrogenation to afford agaritine (**1**) has been described by *Wallcave et al.* [9] who used an *Engelhard*-10% Pd/C catalyst in THF for 24 at r.t. Applying these conditions for the debenzylation of **11** using an *Engelhard*- or *Fluka*-10% Pd/C catalyst resulted only in L-glutamic acid 5-[2-(4-tolyl)hydrazide] (**12**) [8] [9]. The formation of this overreduced product could be avoided only by using a partially poisoned 10% Pd/C catalyst (see *Exper. Part*) in MeOH/H_2O for 35 min at r.t.; this led to 82% of agaritine (**1**).

Conclusions. – The novel synthesis of agaritine (**1**) described here has several advantages as compared to those reported earlier [8] [9]. It affords a higher overall yield (33% based on 4-hydrazinobenzoic acid (**2**)). It avoids the need of purification of intermediates or of the final product by chromatography. It permits the complete characterization of all intermediates by spectroscopy. It passes through the isolated 4-hydrazinobenzylic alcohol (**3**), a compound of probable biological importance [6].

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Experimental Part

1. *General.* Chemicals were *puriss. p.a.* from *Fluka* except DIBAH (*20T*) which was supplied by *Schering*. Anal. TLC: silica gel *60* F_{254} (*Merck 5549* and *Merck 5554*) with solvent systems *A* (petroleum ether 40–65°/EtOAc 8:5), *B* (CH_2Cl_2/MeOH 10:1), or *C* (BuOH/AcOH/H_2O 60:15:25), detection as indicated. Apparatus used: *Bransonic 220* ultrasonic bath; *Christ-Piccolo* centrifuge; *Büchi* melting point apparatus (*Tottoli*), open capillary, m.p. not corrected; *Büchi* rotavapor *RE* (solvent evaporation at 30–35°/18 Torr); *Büchi GKR 50* bulb-to-bulb distillator. Spectra were taken on *Uvikon 810* (UV; λ_{max} (ε) in nm), *Perkin-Elmer 781* (IR; in cm^{-1}; all bands above 1400 cm^{-1} with an intensity > 10% of the background are reported), *Varian XL 200* (^{1}H-NMR; δ in ppm rel. to TMS (= 0 ppm), *J* in Hz[5])), *Varian MAT-711* (MS, *m/z* (% base peak); for *m/z* > 100, all peaks > 5% and for *m/z* < 100, those > 10%; interpretations hypothetical).

2. *Reduction of Methyl 4-Hydrazinobenzoate* (**4**) *with* $LiBH_4$. A soln. of 217 mg (10 mmol) of $LiBH_4$ in 50 ml of THF was added within 20 min to a refluxing soln. of 382 mg (2.3 mmol) of **4** in 50 ml of THF. The reaction was monitored by taking 1.5-ml aliquots from time to time, treating them with 1 ml of H_2O, and subjecting them to anal. TLC (solvent *A*, UV_{254}). After 40 min, **3** (R_f 0.23; *cf. Exper. 3*) and **4** (R_f 0.62) were observed in a ratio of *ca.* 1:4; after 100 min, (4-tolyl)hydrazine (**5**; R_f 0.82) was present in addition to **4** and **3**. After complete disappearance of **4** (15 h reflux), the mixture was cooled to r.t., treated with 20 ml of H_2O and concentrated to *ca.* 30 ml *in vacuo*. The resulting suspension was extracted with 3 × 30 ml of $CHCl_3$. Evaporation of the extract after drying (Na_2SO_4) yielded 240 mg (95%; corrected for the removed 7 aliquots) of *(4-tolyl)hydrazine* (**5**) in clusters of faint yellow needles. M.p. 56–60° ([19]: 66°). UV (H_2O): as in [13]. The hydrochloride, after recrystallization from MeOH/Et_2O, had m.p. 224–225° ([20]: 207–208°).

3. *4-Hydrazinobenzyl Alcohol* (**3**). A stirred soln. of methyl 4-hydrazinobenzoate [8] (**4**; freshly liberated from 1012 mg (5.0 mmol) of its hydrochloride and dried at r.t./10^{-2} Torr) in 100 ml of toluene under Ar was cooled to *ca.* −70° in dry ice/acetone and then treated dropwise with 20 ml of a 20% soln. of DIBAH in toluene (24 mmol) in such a manner that the temp. in the flask did not exceed −65° (*ca.* 45 min). After a further 30 min stirring at −70°, the dry ice/acetone bath was replaced by an ice/NaCl bath, the mixture under Ar treated at 0° dropwise with 50 ml of H_2O[6]) (40 min) and then stirred at 0° for another 30 min. Finally, the two-phase mixture was evaporated and dried at r.t./10^{-2} Torr. All subsequent operations were done under Ar. The colorless solid residue was treated with *ca.* 100 ml of H_2O[6]) and kept in an ultrasonic bath for *ca.* 15 s to facilitate the extraction. The resulting suspension was transferred to 10-ml glass tubes, centrifuged at 4800 r.p.m. for 10 min, and the supernatant decanted through a *G4* sintered glass filter. The residue in each tube was ultrasonized again with *ca.* 5–6 ml of H_2O[6]) and centrifuged and the supernatant decanted and filtered as before. The two supernatants were evaporated together to dryness. The soln. of the residue in *ca.* 25 ml of CH_3CN[6]) was filtered again from some faint precipitate, the clear filtrate evaporated and the slightly yellow residue subjected to bulb-to-bulb distillation at 140° (bath temp.)/$2 \cdot 10^{-4}$ Torr. The distillate solidified immediately yielding 400 mg (58%) of **3** as clusters of colorless needles. M.p. 59–60°. The product could be stored without decomposition under Ar at −5° for at least one week[7]). UV (H_2O): 278 (sh, 2500), 238 (11600). UV (H_2O, 2 drops of 0.1N HCl): 274 (2100), 229 (11500). UV (H_2O, 2 drops of 0.1N HCl followed by 2 drops of 0.1N NaOH): same UV as in H_2O. No change of the UV of $7 \cdot 10^{-5}$ M **3** in KCl/HCl buffer at pH 2.0 during 4 d. IR (CHCl): 3640*m* (OH), 3400*m* (br., NH, NH_2), 3005*m*, 2920*w* (sh), 2880*m*, 1616*s* (aryl), 1515*s*, 1470*w*, 1408*w* (sh). ^{1}H-NMR ($CDCl_3$): 7.26, 6.80 (*AA'MM'*, J_{AM} = 8.5, 4 arom. H); 5.19 (br. *s*, NH or OH); 4.57 (*s*, ArCH_2); 3.56 (br. *s*, NH_2), 1.89 (br. *s*, NH or OH). MS (70 eV): 139 (8, $M^{+\cdot}$ + H), 138 (100, $M^{+\cdot}$), 137 (47), 122 (13), 121 (81, $M^{+\cdot} - H_2O + H$), 120 (8), 107 (18), 104 (15), 94 (23), 93 (11), 92 (36, $M^{+\cdot} - H_2O - N_2$), 91 (16), 79 (13), 78 (17), 77 (45, $C_6H_5^+$), 67 (13), 66 (13), 65 (27), 63 (10), 51 (18). Anal. calc. for $C_7H_{10}N_2O$ (138.17): C 60.85, H 7.30, N 20.27; found: C 60.89, H 7.27, N 20.28.

4. *$LiAlH_4$ Reduction of* **3**. A soln. of 20 mg (0.15 mmol) of **3** in 10 ml of Et_2O was added dropwise to a soln. of 7.5 mg (0.20 mmol) of $LiAlH_4$ in 5 ml of Et_2O without external cooling. After refluxing for 5 h, 5 ml of EtOAc and then 10 ml of H_2O were added. After separation of the org. layer and further extraction of the aq. phase with 2 × 10 ml of Et_2O, the combined org. solutions were dried (K_2CO_3) and evaporated to yield 17 mg (93%) of **5** as a colorless solid. M.p. 58–62° ([19]: 66°). Anal. TLC (solvent *A*, UV_{254}): R_f 0.82, cochromat. with authentic **5**.

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[5]) The number of protons in the assignment corresponds always within ± 10% to the integration.

[6]) Solvents were degassed by keeping them, prior to use, in an ultrasonic bath for 5 min.

[7]) Crystalline **3** when kept in contact with air became yellow to orange (at r.t. within a few min, at −5° within some h). Anal. TLC (solvent *A*, UV_{254}) of the colored material showed at least 6 spots including that of **3** at R_f 0.23.

5. *Oxidation of* **3** *with* SeO_2. A stirred soln. of 14 mg (0.1 mmol) of **3** in 1.5 ml of 1N HCl was cooled in an ice bath and treated with 12 mg (0.11 mmol) of SeO_2. The resulting suspension was centrifuged at 4000 r.p.m. and the clear supernatant treated with a soln. of 15 mg (0.1 mmol) of 2-naphthol in 0.75 ml of H_2O with cooling, neutralized with 5% NaOH soln. and the precipitate, after filtration, recrystallized from EtOH/H_2O to yield 25 mg (90%) of *2-hydroxynaphthalene-1-azo-4'-methanolbenzene* (**9**) as orange needles. M.p. 171° ([14]: 162–163°). UV (95% EtOH): 483 (17200), 418 (12570), 310 (8640), 229 (32780) ([14]: 482 (15500), 228 (32400)). IR ($CHCl_3$): 3600*m* (OH), 3080*m*, 3000*m*, 1620*s* (aryl), 1600*m*, 1552*m*, 1505*s*, 1450*s*, 1429*m*, 1404*m*. MS: as in [14].

6. *1-Benzyl 5-Hydrogen* N-*(Benzyloxycarbonyl)*-L-*glutamate* (**10**). A suspension of 1.0 g (1.8 mmol) of the dicyclohexylamine salt of **10** [17] in 20 ml of 1.0N HCl was stirred vigorously overnight. The resulting suspension of finely dispersed colorless crystals was extracted once with 100 ml of CH_2Cl_2 and washed with *ca.* 30 ml of H_2O. The org. phase, after drying (Na_2SO_4), was evaporated. The oily residue solidified within 2–3 days to yield 630 mg (94%) of ^{1}H-NMR-pure **10** as an amorphous colorless solid. M.p. 64–66°. A portion was crystallized from $CHCl_3$/petroleum ether (40–65°) to obtain anal. pure **10** as fine needles. M.p. 67–68°. UV (EtOH): 267 (206), 263 (315), 257 (381), 219 (1070). IR (KBr): 3600–3100*m* (br., OH), 3342*m* (NH), 1740*s* and 1730*s* (ester and carbamate C=O), 1660*s* (carboxylic C=O), 1552*m* (sh), 1550*m*, 1500*w*, 1455*w*, 1450*m*, 1410*m*. ^{1}H-NMR ($CDCl_3$): 10.63 (br. *s*, OH, exchangeable with D_2O); 7.5–7.1 (*m*, 2 C_6H_5); 5.52 (*d*, J = 7.8, NH, slowly exchangeable with D_2O); 5.15, 5.09 (2 *s*, 2 ArCH_2); 4.6–4.4 (*m*, H–C(2)); 2.5–2.35 (*m*, CH_2(4)); 2.3–2.1, 2.1–1.9 (2 *m*, ratio 1:1, CH_2(3)). ^{1}H-NMR ((D$_6$) DMSO): 12,19 (br. *s*, OH, exchangeable with D_2O); 7.80 (*d*, J = 8.0, NH, exchangeable with D_2O); 7.6–7.2 (*m*, 2 C_6H_5), 5.13, 5.04 (2 *s*, 2 ArCH_2); 4.25–4.05 (*m*, H–C(2)); 2.45–2.25 (*m*, CH_2(4)); 2.1–1.7 (*m*, CH_2(3)). Anal. calc. for $C_{20}H_{21}NO_6$ (371.37): C 64.68, H 5.70, N 3.77; found: C 64.79, H 5.43, N 3.63.

7. N^2-*(Benzyloxycarbonyl)*-L-*glutamic Acid 1-(Benzyl Ester) 5-*{*2-[4-(Hydroxymethyl)phenyl]hydrazide*} (**11**). A soln. of 371 mg (1.0 mmol) of **10** in 100 ml of CH_2Cl_2 was thoroughly evacuated and flushed with Ar. Maintaining under Ar, the soln. was stirred, cooled to *ca.* −10° and then treated dropwise with a soln. of 206 mg (1.0 mmol) of DCC in 30 ml of CH_2Cl_2 (15 min). After stirring at −10° for further 4 h, the anal. TLC (solvent *B*, 25% H_2SO_4 in EtOH/140°) showed complete disappearance of **10** (R_f 0.50) and instead a new spot (R_f 0.25). Then a soln. of 138 mg (1.0 mmol) of freshly distilled **3** in 50 ml of CH_2Cl_2 was added dropwise (25 min) and the mixture stirred for another 1.5 h at −10°, when the ice-bath was removed and stirring continued for further 1.5 h (→ r.t., clear mixture). The solvent was evaporated, the residue treated with *ca.* 30 ml of CH_2Cl_2, filtered, and the clear filtrate evaporated again. This residue was treated with *ca.* 15 ml of THF, warmed gently (*ca.* 50°), and the slightly turbid soln. filtered through a *G4* sintered glass. The pale yellow filtrate was treated with excess Et_2O and allowed to stand overnight at −20° for crystallization. The precipitate was filtered, washed with a little Et_2O and dried at r.t./10^{-2} Torr: 400 mg (81%) of **11** as a pale yellow amorphous solid. M.p. 126–127° ([9]: 123–125°). UV (EtOH): 284 (1320), 240 (11100). IR (KBr): 3310*s* (br.), 3060*w*, 3030*w*, 2925*m*, 2850*m*, 1740*m* (sh) and 1732*m* (ester and carbamate C=O), 1693*s* (hydrazide C=O), 1648*m*, 1629*m*, 1612*m* (aryl), 1538*m*, 1515*m*, 1452*m*. ^{1}H-NMR ((D$_6$)DMSO, 22°): 9.62 (*d*, J = 2.6, NH-C(5), exchangeable with D_2O); 7.86, 7.76 (2 *d*, J = 8.0, ratio *ca.* 85:15, NH-C(2) of 2 amide rotamers, slowly exchangeable with D_2O); 7.61 (*d*, J = 2.6, ArNH, exchangeable with D_2O); 7.5–7.2 (*m*, 2 C_6H_5); 7.05, 6.63 (*AA'MM'*, J_{AM} = 8.3, H–C(3'), H–C(5'), H–C(2'), H–C(6')); 5.15 (*s*, CH_2OC(1)); 5.08, 5.06 (2 *s*, ratio *ca.* 1:4, CH_2OCONHC(2) of 2 amide rotamers); 4.90 (*t*, J = 5.5, CH_2OH, exchangeable with D_2O); 4.33 (*d*, J = 5.5, CH_2OH, *s*, after treatment with D_2O); 4.25–4.05 (*m*, H–C(2)); 2.45–2.20 (*m*, CH_2(4)); 2.2–1.95, 1.95–1.70 (2 *m*, ratio 1:1, CH_2(3)). ^{1}H-NMR ((D$_6$)DMSO, 40°): same ^{1}H-NMR as at 22°, except that 7.86 and 7.76 (2 *d*, J = 8.0, NH–C(2)) were replaced by 7.60 (*d*, J = 8.0), and 5.08 and 5.06 (2 *s*, CH_2OCONHC(2)) were replaced by 5.06 (*s*). ^{1}H-NMR ((D$_6$)DMSO, 60°): same ^{1}H-NMR as at 40°, except that 7.60 (*d*, J = 8.0, NH–C(2)) was replaced by 7.63 (br. *s*). MS (70 eV): 491 (4, $M^{+\cdot}$), 211 (6), 138 (8), 137 (28), 121 (13), 107 (20), 91 (100), 79 (18), 77 (12). Anal. calc. for $C_{27}H_{29}N_3O_6$ (491.52): C 65.98, H 5.95, N 8.55; found: C 65.97, H 5.70, N 8.34.

8. *Agaritine* (**1**). A soln. of 0.1 ml of a quinoline-sulfur catalyst poison[8]) prepared according to [21] in 10 ml of MeOH and 10.0 mg of 10% Pd/C were added to a soln. of 49 mg (0.1 mmol) of **11** in 50 ml of MeOH/H_2O 9:1 *(v/v)*. After stirring the suspension for 2 min, H_2 gas was applied from a balloon on the top of the flask with stirring for 35 min. Then, the mixture was filtered, the filtrate evaporated, and the residue dissolved in a minimum of H_2O

[8]) Attempts to dissolve the catalyst poison in xylene as described in [21] resulted in a suspension. After standing overnight, a sediment had separated. The clear supernatant, after dilution with a 100 fold volume of MeOH, was used as catalyst poison. Its poisoning activity diminished with time. Therefore, to avoid overreduction of **11** to L-*glutamic acid 5-[2-(4-tolyl)hydrazide]* (**12**), it is recommended to test the poison activity prior to use in anal. reduction runs of **11**. Formation of **12** aside from **1** is easily followed by anal. TLC of the reaction mixture (solvent *C*, ninhydrin; R_f of **1**, 0.36, R_f of **12**, 0.24).

and treated with an equal amount of EtOH and then with CH_3CN to afford precipitation. After standing overnight, the precipitate was filtered off and dried at r.t./10^{-2} Torr to yield 22.0 mg (82%) of **1** as a colorless powder. M.p. 196–198° ([8]: 205–208°). UV: as in [2] [8]. IR (KBr): 3420*s* (br., OH), 3260*m* (br., NH), 3100–3000*w* (br., NH_3^+), 2920*m*, 1660*s* (NH_3^+, amino-acid I), 1615*s* (aryl), 1585*m* (CO_2^-), 1512*s* (NH_3^+, amino-acid II), 1410*m*. ^{1}H-NMR (D_2O): 7.14, 6.73 (*AA'MM'*, J_{AM} = 8.6, 4 arom. H); 4.38 (*s*, ArCH_2); 3.8–3.6 (*m*, H–C(2)); 2.5–2.3 (*m*, CH_2(4)); 2.2–1.9 (*m*, CH_2(3)). FAB-MS: as in [3]. Anal. calc. for $C_{12}H_{17}N_3O_4$ (267.27): C 53.92, H 6.41, N 15.72; found: C 53.66, H 6.60, N 15.54.

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