

84. A New Synthesis of Coprine and *O*-Ethylcoprine

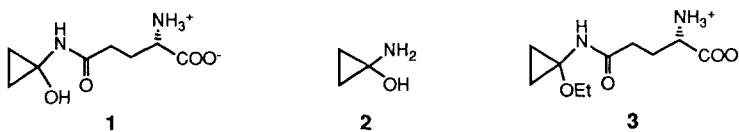
by Thomas Kienzler, Peter Strazewski, and Christoph Tamm*

Institut für Organische Chemie der Universität Basel, St.-Johanns-Ring 19, CH-4056 Basel

(25.III.92)

Coprine (**1**), a toxin of the mushroom *Coprinus atramentarius*, was synthesized starting from the 2-amino- and 1-carboxy-protected L-glutamic acids **4** and **12**. Compound **4** was first decarboxylated by a radical chain reaction to bromide **5** which underwent ring closure to cyclopropanecarboxylate **6** on treatment with NaH (*Scheme 1*). Subsequent oxidative electrolysis of **7** to form *tert*-butyl *N*-(1-ethoxycyclopropyl)carbamate (**8**) and acidic hydrolysis yielded the 1-aminocyclopropanol hydrochloride (**9**). Selective cleavage of the amino-protecting group of **8** (\rightarrow **10** or **11**), coupling of the corresponding amine **13** with L-glutamic acid **12**, and acidic hydrolysis of the resulting L-glutamine derivative **17** yielded *O*-ethylcoprine (**3**) and coprine (**1**).

Introduction. – The common inky cap (*Coprinus atramentarius* BULL.) is an edible and palatable mushroom which has been known for many years to cause a severe oversensitivity to EtOH after consumption [1]. The symptoms of poisoning occurring after drinking alcoholic beverages with or after a meal that includes *C. atramentarius* are similar to that of the drug disulphiram (*Antabus*[®]) [2]. In 1975, the causative agent named coprine (**1**), was isolated from the fruiting body of *C. atramentarius* independently by two groups [3]. Subsequent biochemical and pharmacological studies showed that coprine is a potent *in vivo* inhibitor of the NAD⁺-dependent aldehyde dehydrogenase (ALDH; EC 1.2.1.3), whereas the product of metabolic hydrolysis of the γ -carboxamide group, 1-aminocyclopropanol (**2**), inhibits ALDH *in vivo* and *in vitro* [4].



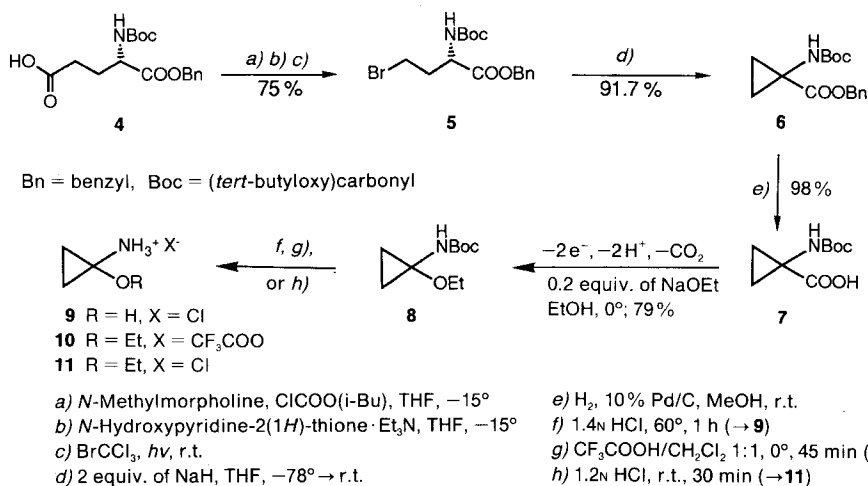
Two years after its isolation, Lindberg *et al.* confirmed the structure of coprine (= *N*⁵-(1'-hydroxycyclopropyl)-L-glutamine; **1**) by reporting a synthetic route yielding the natural product and some related cyclopropanone derivatives [5]. Its congener *O*-ethylcoprine (= *N*⁵-(1'-ethoxycyclopropyl)-L-glutamine; **3**) showed a similar effect on ALDH as coprine and proved to be very useful for recent studies on alcohol metabolism [6].

Here, we report a new synthesis of coprine (**1**) and *O*-ethylcoprine (**3**) and the details of the synthesis of 1-aminocyclopropanol (**2**) that was already reported in a preliminary communication [7].

Results and Discussion. – First, the development of a new synthesis for the *N,O*-disubstituted cyclopropane moiety comprising the structural and pharmacological key feature of coprine (**1**) and *O*-ethylcoprine (**3**) was envisaged. The intuitively simplest starting material for the synthesis of cyclopropanone hemiaminal derivatives would probably be cyclopropanone itself. Unfortunately, several investigations showed that these hemiaminals are too reactive to be trapped and isolated in sufficient amounts [8]. An alternative approach implies the degradation of larger molecules to the desired size. We, therefore, decided to use an 1-aminocyclopropane-1-carboxylic-acid derivative as starting material.

A simple and high-yielding synthesis of 1-aminocyclopropane-1-carboxylic acid [9] starting from the commercially available 2-amino- and 1-carboxy-protected L-glutamic acid **4** (Scheme 1) afforded a suitable precursor, *i.e.* 1-[(*tert*-butyloxy)carbonylamino]-cyclopropane-1-carboxylic acid (**7**). Acid **4** was decarboxylated by a radical chain reaction in the presence of BrCCl_3 to bromide **5** which, upon treatment with NaH , underwent γ -elimination to cyclopropanecarboxylate **6**. Hydrogenolytic debenzoylation furnished **7** in up to 68% overall yield from **4**.

Scheme 1

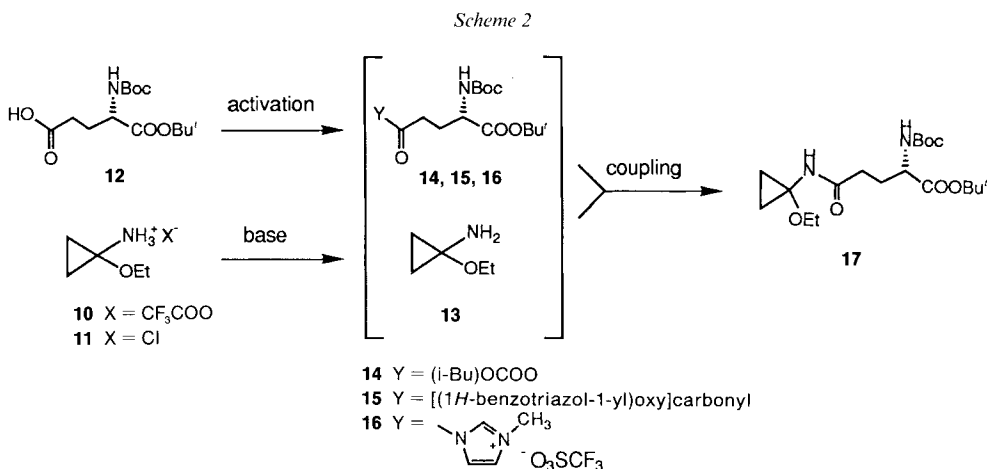


The decarboxylation of acid **7** was investigated next. The oxidative radical chain reaction using tris(phenylthio)antimony, air (O_2), and H_2O to form the nor-alcohol, as reported by Barton *et al.* [10], was not successful, even when applied to the more stable model compound 2-[(*tert*-butyloxy)carbonylamino]isobutyric acid (Boc-Aib); although decarboxylation took place, the product was unstable under the conditions used and fragmented into *tert*-butyl carbamate and acetone [11]. However, anodic oxidative decarboxylation of **7** according to Hofer and Moest [12] gave the corresponding cyclopropanone *N*-acyl *O*-alkyl hemiaminal **8** in 79% yield (Scheme 1). This type of two-electron oxidation is known to provoke particularly *N*-acylated α -amino acids to decarboxylate; efficient substitution of the well stabilized intermediate acyliminium ion by a nucleo-

phile takes place under exceptionally mild conditions [13]. In the present case, replacement of COOH by OEt was achieved with EtOH acting as solvent and nucleophile. Comparison of the physical and spectral data of product **8** with published data for **8** obtained by photochemical synthesis [14] confirmed the structure.

Hydrolysis of **8** with 1.4*N* HCl at 60° led to the crystalline, but hygroscopic hydrochloride **9**, a stabilized form of 1-aminocyclopropanol (**2**), in 86% yield [7]. Many reagents were recommended in the literature for the selective cleavage of the acid-labile Boc protection [15]. The removal with CF₃COOH/CH₂Cl₂ 1:1 at 0° yielded trifluoroacetate **10** in 89% yield and deprotection with 1.2*N* HCl in AcOH at room temperature the corresponding hydrochloride **11** (92%).

The next step in the synthesis of coprine (**1**) and *O*-ethylcoprine (**3**) was the coupling of acid **12** and the labile amine **13**. The latter had to be generated *in situ* from the ammonium salts **10** or **11** by adding 1 equiv. of base (Scheme 2). Several electron-withdrawing groups Y were tested for the activation of the carboxyl group in **12**. First, the



classical mixed-anhydride method was carried out using **14** (obtained from **12** by isobutyl chlorocarbonate treatment in THF at -15° [16]) to which **13** (obtained from **10** and Et₃N in dimethylformamide at room temperature) was added dropwise and stirred for 30 min at -15° . After 2 h at room temperature and chromatography, the desired coupling product **17** was obtained in a yield of only 19%. Activation of **12** with dicyclohexylcarbodiimide (DCC) and 1*H*-benzotriazol-1-ol (BtOH) [17] (\rightarrow **15**) and generation of **13** from **11** (in THF at 0°) with *Hünig's* base resulted in 8.5% of pure **17**, after multiple chromatographic purifications to remove the dicyclohexylurea formed on activation. Using [(1*H*-benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as activating reagent [18] (\rightarrow **15** + hexamethylphosphoric triamide) and *in situ* generation of **13** from **11** with Et₃N, the coupling yield after 18 h at room temperature was still low (18%); presumably, amine **13** decomposed faster than it substituted the activating group Y. Therefore, the highly reactive reagent 1,1'-carbonylbis(3-methylimidazolium) triflate, developed by *Rapoport* and coworkers [19], was used to activate **12**,

and the cationic intermediate **16** and amine **13** (generated *in situ* from **10**) gave coupling product **17** in 28% yield in 2 h at 10°. As no alternative fast coupling reagents are known to be useful for sterically hindered amines, no further attempts were made to improve this step of the synthesis.

The Boc and *t*-Bu protecting groups of **17** were removed using 1.2M HCl at 40°, and after ion-exchange chromatography, the pharmacologically interesting *O*-ethylcoprine (**3**) was obtained in 78.5% yield. Under more rigorous acidic conditions at 60°, the EtO group was also hydrolyzed to give coprine (**1**) in 73% yield. The physical and spectral data of **1** and **3** were in good agreement with the data reported in [5], and the 400-MHz ¹H-NMR spectra of the mixture of synthetic and natural **1** supported the established structure [11]. Thus, coprine (**1**) and *O*-ethylcoprine (**3**) were obtained in 9.7 and 10.5% overall yield, respectively, starting from both commercially available L-glutamic acids **4** and **12**.

We gratefully acknowledge the gift of a sample of natural coprine by Prof. *B. Wickberg*, Lund Institute of Technology, Sweden, and the support of this investigation by the *Swiss National Science Foundation*.

Experimental Part

General. All reagents and chemicals were purified and dried by standard procedures. Electrochemistry: potentiostat/galvanostat (62 V/max. 1 A) and Pt electrodes (5.5 cm²) were used in an undivided cell with cooling and under slight stirring. THF was distilled over Na/K prior to use and transferred with syringes. TLC: *Alugram SIL G/UV₂₅₄* (*Macherey-Nagel*), *RP-18 F₂₅₄* (*Merck*), aluminium oxide *UV₂₅₄* (*Fluka*), and cellulose *UV₂₅₄* (*Fluka*) plates; detection under UV light where possible; by I₂, or with 5% (*v/v*) H₂SO₄ in MeOH or 2% (*w/v*) ninhydrine in EtOH-followed by heating. Column chromatography (CC): silica gel 60 (*Merck*, 0.063–0.200 mm or 0.040–0.063 mm). Flash chromatography (FC): silica gel 60 (*Chemische Fabrik Uetikon*, 0.030–0.075 mm). Ion-exchange chromatography: *Amberlite CG-120-II* (*Fluka*; strong cation-exchange resin) and *Amberlite CG-400-II* (*Fluka*; strong anion-exchange resin). M.p.: *Kofler* block; corrected. [α]_D: *Perkin-Elmer-141* polarimeter (1-dm cell). IR: *Perkin-Elmer-781* spectrometer; $\bar{\nu}$ in cm⁻¹. NMR: *Varian-EM-360* (¹H, 60 MHz), *Varian-Gemini-300* (¹H, 300 MHz; ¹³C, 75 MHz), or *Varian-VXR-400* (¹H, 400 MHz; ¹³C, 101 MHz) spectrometer; δ in ppm downfield of TMS (=0.00 ppm) or rel. to sodium 3-(trimethylsilyl)propionate (δ (H) 0.00 ppm, δ (C) 1.70 ppm) for D₂O solns.: coupling constants *J* in Hz. MS: *VG 70-250*; in *m/z* (rel. intensity (%)).

Benzyl 4-Bromo-2-[(tert-butyloxy)carbonylamino]butanoate (5). To a soln. of 1-benzyl *N*-[(*tert*-butyloxy)carbonyl]-L-glutamate (5.0 g, 14.83 mmol; **4**; *Novabiochem*) in THF (75 ml), *N*-methylmorpholine (1.65 ml, 14.83 mmol) and isobutyl chlorocarbonate (95%; 2.05 ml, 14.83 mmol; *Fluka*) were added successively at –15°. The mixture was stirred for 5 min, a soln. of 1-hydroxypyridine-2(*1H*)-thione (2.26 g, 17.80 mmol) and Et₃N (2.48 ml, 17.80 mmol) in THF (50 ml) added dropwise, and the heterogeneous mixture stirred for 30 min at –15°. After filtration of the *N*-methylmorpholinium chloride, the filtrate was evaporated and the resulting yellow oil dissolved in BrCCl₃ (146 ml, 1.483 mol) and irradiated for 45 min at r.t. with a 125-W tungsten lamp. The brownish mixture was evaporated and the residue purified by FC (pentane/CH₂Cl₂ 2:3, then CH₂Cl₂): 4.137 g (75.0%) of **5**. The colorless crystals were recrystallized from pentane. M.p. 53–55° ([α]_D: 53°). IR (KBr): 3380s (NH), 3040w (CH), 3020w (CH), 2990m (CH), 2950w, 1765s (CO), 1685s (NCO I), 1515s (NCO II), 1440m, 1370m, 1295m, 1250m, 1155s. ¹H-NMR (400 MHz, CDCl₃): 1.44 (*s*, *t*-Bu); 2.22 (*m*, CH₂(3)); 2.42 (*m*, CH₂(4)); 3.40 (*dt*, *J* = 5.2, 2, H–C(2)); 5.11 (*br. d*, *J* = 2, NH); 5.19 (*d*, *J* = 2.8, PhCH₂); 7.26–7.40 (*m*, 5 arom. H). CI-MS (NH₃): 391, 389 (0.8, 0.9, [*M* + NH₄]⁺); 374, 372 (1.4, 1.4, [*M* + H]⁺); 335, 333 (23.5, 24, [*M* – (*t*-Bu) + NH₄]⁺); 274, 272 (97.5, 100, [*M* – Boc]⁺); 236 (12); 192 (14); 138, 136 (18.5, 19.5); 108 (35); 91 (32); 56 (33). Anal. calc. for C₁₆H₂₂BrNO₄ (372.26): C 51.62, H 5.96, N 3.76, Br 21.47; found: C 51.39, H 6.03, N 3.75, Br 21.54.

Benzyl 1-[(tert-Butyloxy)carbonylamino]cyclopropane-1-carboxylate (6). NaH (55–60% in oil; 58.6 mg, \geq 1.34 mmol) was twice suspended in hexane (5 ml) and the gray soln. removed carefully. The pure NaH under Ar was suspended in THF (10 ml) and cooled to –78°. Slowly, **5** (250 mg, 0.67 mmol) in THF (5 ml) was added dropwise. After the addition (30 min), the mixture was allowed to gradually warm up to r.t., stirred for 5 h at r.t., and then treated with sat. NH₄Cl soln. (20 ml) and extracted with CH₂Cl₂ (3 \times 25 ml). The combined org. phase

was washed with brine (20 ml), dried (Na_2SO_4), and evaporated: 179 mg (91.7%) of **6**. The colorless crystals were recrystallized from (i-Pr)₂O. M.p. 116–118°. IR (KBr): 3350s (NH), 3030w (CH), 3020w (CH), 2995m (CH), 2940w, 1740s (CO), 1690s (NCO I), 1515s (NCO II), 1450m, 1360m, 1300s, 1270m, 1190s, 1150s, 755s, 695m. ¹H-NMR (400 MHz, CDCl_3): 1.12–1.22 (m, 2H, $\text{CH}_2(2), \text{CH}_2(3)$); 1.42 (s, *t*-Bu); 1.52–1.64 (m, 2H, $\text{CH}_2(2), \text{CH}_2(3)$); 5.31 (s, PhCH_2); 5.17–5.20 (br. s, NH); 7.28–7.42 (m, 5 arom. H). CI-MS (NH_3): 3.09 (2.4, $[\text{M} + \text{NH}_4]^+$); 292 (3, $[\text{M} + \text{H}]^+$), 253 (42.5, $[\text{M} - (t\text{-Bu}) + \text{NH}_4]^+$), 235 (23, $[\text{M} - (t\text{-Bu})]^+$), 218 (10.5), 192 (100, $[\text{M} - \text{Boc}]^+$), 108 (62.5), 91 (45), 58 (10.5). Anal. calc. for $\text{C}_{16}\text{H}_{21}\text{NO}_4$ (291.35): C 65.96, H 7.26, N 4.81; found: C 66.11, H 7.30, N 4.62.

1-[(tert-Butyloxy)carbonylamino]cyclopropane-1-carboxylic Acid (7). To a soln. of **6** (1.75 g, 6.0 mmol) in MeOH (30 ml), 10% Pd/C (175 mg; *Fluka*) were added. The mixture was hydrogenated at r.t./1 atm. After 90 min (calc. amount of H_2 (146.5 ml) consumed), the mixture was filtered through *Celite 535* and the filtrate evaporated: 1.185 g (98%) of **7**. Colorless crystals. M.p. 176–177.5°. IR (KBr): 3600–2750s (br., OH), 3300s (NH), 3250s (NH), 3100m, 3030w (CH), 3010w (CH), 2980m (CH), 2960m, 2850m, 1700s (br., CO, NCO I), 1645s (NCO II), 1485m, 1410s, 1370s, 1300s, 1200s, 1160s, 1080s, 930m, 780m. ¹H-NMR (60 MHz, CD_3OD): 0.8–1.2 (m, 2H, $\text{CH}_2(2), \text{CH}_2(3)$); 1.25–1.5 (m, 2H, $\text{CH}_2(2), \text{CH}_2(3)$); 1.4 (s, *t*-Bu); 4.7–5.2 (br. s, NH). CI-MS (NH_3): 219 (1.9, $[\text{M} + \text{NH}_4]^+$), 202 (2.7, $[\text{M} + \text{H}]^+$), 163 (50.5, $[\text{M} - (t\text{-Bu}) + \text{NH}_4]^+$), 145 (53.7, $[\text{M} - (t\text{-Bu})]^+$), 119 (6.1), 102 (100, $[\text{M} - \text{Boc}]^+$), 86 (8), 58 (12.7). Anal. calc. for $\text{C}_9\text{H}_{15}\text{NO}_4$ (201.22): C 53.72, H 7.51, N 6.96; found: C 53.65, H 7.60, N 6.85.

tert-Butyl N-(1-Ethoxycyclopropyl)carbamate (8). To a soln. of **7** (0.402 g, 2.0 mmol) in abs. EtOH (50 ml) in an undivided electrolytic cell with Pt-electrodes, 0.98M NaOEt in EtOH (408 μl , 0.4 mmol) were added at 0°. The electrolysis was performed under light stirring and a constant current of $i = 9 \text{ mAcm}^{-2}$. After 7 h (3.1 Fmol^{-1}), TLC (Al_2O_3 , CH_2Cl_2) showed a uniform product (R_f 0.3). The current was switched off, and strong acidic cation-exchange resin (*Dowex 50 W* $\times 8$, H^+ -form, 20–50 mesh; 2 g) was added. After 10 min, the neutralized mixture was filtered, the filtrate evaporated, and the oily residue purified by CC (Al_2O_3 , CH_2Cl_2 , then $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ 10:1): 317 mg (79%) of **8**. Colorless needles. M.p. 41–42° ([14]: 44–45°). IR (KBr): 3360s (NH), 2990s (CH), 2965m (CH), 1710s (br., NCO I), 1520s (NCO II), 1400m, 1370s, 1270m, 1240m, 1070s, 950m, 855m. ¹H-NMR (400 MHz, CDCl_3): 0.94–0.99 (m, 2H, $\text{CH}_2(2), \text{CH}_2(3)$); 1.07–1.14 (m, 2H, $\text{CH}_2(2), \text{CH}_2(3)$); 1.18 (t, $J = 7.2$, $\text{CH}_3\text{CH}_2\text{O}$); 1.47 (s, *t*-Bu); 3.64 (q, $J = 7.2$, 2H, $\text{CH}_3\text{CH}_2\text{O}$); 5.4–5.6 (br. s, NH). CI-MS (NH_3): 219 (14.4, $[\text{M} + \text{NH}_4]^+$), 202 (17.7, $[\text{M} + \text{H}]^+$), 163 (100, $[\text{M} - (t\text{-Bu}) + \text{NH}_4]^+$), 146 (45.0, $[\text{M} - (t\text{-Bu}) + \text{H}]^+$), 102 (14.0, $[\text{M} - \text{Boc}]^+$), 73 (21.3), 56 (18.9). Anal. calc. for $\text{C}_{10}\text{H}_{19}\text{NO}_3$ (201.27): C 59.68, H 9.52, N 6.96; found: C 58.98, H 9.70, N 6.85.

1-Aminocyclopropanol Hydrochloride (9). A suspension of **8** (166 mg, 0.825 mmol) in aq. 1.4M HCl (10 ml) was heated to 60° for 1 h and stirred vigorously. To the resulting homogeneous mixture (TLC (SiO_2 , AcOH/BuOH/ H_2O 1:3:1); R_f 0.51 (pure)), H_2O (10 ml) was added and the soln. evaporated. To remove traces of HCl, the addition of H_2O (10 ml) followed by evaporation was repeated 3 times. Drying at 50°/0.02 Torr yielded 77 mg (86%) of **9**. Colorless, hygroscopic salt. IR (KBr): 3600–3200s (br., OH ass.), 3300–2500s (br., NH_3^+), 2990s (CH), 1720w, 1610w, 1450w, 1350m, 1250s (C–O), 1170w, 1120w. ¹H-NMR (60 MHz, D_2O): 1.1–1.2 (br. m, $\text{CH}_2(2), \text{CH}_2(3)$). FAB-MS (glycerine): 166 (30.1, $[\text{M} + \text{glycerine}]^+$), 132 (4.4, $[\text{C}_3\text{H}_4 + \text{glycerine}]^+$), 104 (30.4), 74 (100, M^+), 61 (1.4), 57 (1.5, $[\text{M} - \text{OH}]^+$).

1-Ethoxycyclopropylammonium Trifluoroacetate (10). To a soln. of **8** (99 mg, 0.493 mmol) in abs. CH_2Cl_2 (0.8 ml), CF_3COOH (0.8 ml, 10 mmol) was added at 0° and the homogeneous soln. stirred vigorously. After 45 min (TLC (SiO_2 , $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ 95:5): no **8** at R_f 0.49), the mixture was dissolved in AcOEt (10 ml) and evaporated. To remove traces of CF_3COOH , the addition of AcOEt (10 ml) followed by evaporation was repeated twice. Drying at r.t./0.01 Torr for 6 h yielded 94 mg (89%) of **10**. Partly crystalline salt. ¹H-NMR (300 MHz, (D_6)DMSO): 0.85–1.32 (m, $\text{CH}_2(2), \text{CH}_2(3)$); 1.24 (t, $J = 7.2$, $\text{CH}_3\text{CH}_2\text{O}$); 3.67 (q, $J = 7.2$, $\text{CH}_3\text{CH}_2\text{O}$); 8.35–9.05 (br. s, NH_3^+). EI-MS (70 eV): 102 (0.3, M^+), 95 (5.0), 87 (2.7, $[\text{M} - \text{CH}_3]^+$), 86 (5.6), 73 (7.4), 69 (85.1, $[\text{M} - \text{CH}_3 - \text{NH}_4]^+$), 56 (22.8, $[\text{M} - \text{EtO}]^+$), 51 (43.9), 45 (100, EtO^+).

1-Ethoxycyclopropylamine Hydrochloride (11). A suspension of **8** (101 mg, 0.502 mmol) in 1.2N HCl/AcOH (1.8 ml, 2 mmol) was stirred for 30 min at r.t. (TLC (SiO_2 , AcOH/BuOH/ H_2O 1:3:1): R_f 0.40). After evaporation, AcOH (2 ml) was added twice to remove traces of HCl. Evaporation and drying at r.t./0.01 Torr for 18 h gave 63.8 mg (92.3%) of **11**. Partly crystalline salt. ¹H-NMR (300 MHz, CDCl_3): 0.85–1.35 (m, $\text{CH}_2(2), \text{CH}_2(3)$); 1.25 (t, $J = 7.3$, $\text{CH}_3\text{CH}_2\text{O}$); 3.62 (t, $J = 7.3$, $\text{CH}_3\text{CH}_2\text{O}$); 8.7–9.5 (br. s, NH_3^+); traces of AcOH visible. EI-MS (70 eV): 99 (4.4, $[\text{M} - 3\text{H}]^+$), 83 (2.5, $[\text{M} - 2\text{H} - \text{NH}_3]^+$), 69 (15.2, $[\text{M} - \text{CH}_3 - \text{NH}_4]^+$), 60 (36.7), 56 (98.0, $[\text{M} - \text{EtO}]^+$), 45 (56.2, EtO^+), 43 (100, Ac^+ from AcOH), 41 (26.4, C_3H_5^+).

tert-Butyl (2S)-N²-[(tert-Butyloxy)carbonylamino]-N⁵-(1'-ethoxycyclopropyl)glutamate (17). $\text{CF}_3\text{SO}_3\text{Me}$ (255 μl , 2.32 mmol; *purum, Fluka*) was added dropwise *via* syringe to a soln. of 1,1'-carbonylbis(imidazole) (188 mg, 1.16 mmol) in abs. nitromethane (2 ml) at 10°. To this freshly generated soln. of 1,1'-carbonylbis(3-methylimi-

dazolium) triflate, a mixture of *tert*-butyl *N*-[(*tert*-butyloxy)carbonyl]-L-glutamate (351.3 mg, 1.16 mmol; **12**; Novabiochem) and *N*-methylimidazole (99%; 10 μ l, 0.12 mmol; Fluka) dissolved in abs. nitromethane (0.5 ml) and abs. DMF (0.1 ml) was added *via* syringe. After 10 min, when CO₂ evolution ceased, a soln. of **10** (125 mg, 0.58 mmol) in abs. DMF (1.5 ml) was added and the mixture stirred for a few min. Then *N*-methylmorpholine (64 μ l, 0.58 mmol) was added to generate **13** *in situ*, and the mixture was stirred for 2 h at 10°. Then, H₂O (5 ml) was added, followed by AcOEt (30 ml). After successive extraction with sat. aq. NaHCO₃ soln. (30 ml) and H₂O (30 ml), the combined org. phase was dried (Na₂SO₄) and evaporated. The yellow oily residue was purified by CC (SiO₂, pentane/Et₂O 1:3): 62.8 mg (28%) of **17**. The colorless crystals were recrystallized from pentane. M.p. 54–55°. [α]_D²⁰ = –23.4 (*c* = 1.02, MeOH). IR (KBr): 3350s (NH), 3290s (NH), 3010w (CH), 2990s (CH), 2940m (CH), 1730s (br., CO), 1695s (NCO I), 1545s (br., NCO II), 1450m, 1390m, 1370s, 1300s (br.), 1250m, 1155s (br.), 1065m, 1025m, 850m. ¹H-NMR (300 MHz, CDCl₃): 0.91–0.98 (*m*, 2H, CH₂(2'), CH₂(3')); 1.07–1.77 (*m*, 2H, CH₂(2'), CH₂(3')); 1.23 (*t*, *J* = 6.9, CH₃CH₂O); 1.44, 1.46 (2s, 2 *t*-Bu); 2.02–2.33 (br. *m*, CH₂(3), CH₂(4)); 3.64 (*q*, *J* = 6.9, CH₃CH₂O); 4.08–4.27 (*m*, H–C(2)); 5.24–5.38 (br. *m*, NH–C(2)); 7.14–7.23 (br. *s*, NH–C(5)). ¹³C-NMR (101 MHz, CDCl₃): 14.31, 14.45 (C(2'), C(3')); 15.30 (CH₃CH₂O); 27.87, 28.20 (2 (CH₃)₂C); 29.49 (C(3)); 32.78 (C(4)); 53.51 (C(2)); 62.73 (CH₃CH₂O); 65.50 (C(1')); 79.84, 82.14 (2 (CH₃)₂C); 155.86 (NCOO(*t*-Bu)); 171.37, 172.55 (C(5), C(1)). CI-MS (NH₃): 387 (98.3, [*M* + H]⁺), 341 (10.3, [*M* – EtO]⁺), 331 (100, [*M* – (*t*-Bu)]⁺), 287 (53.3, [*M* – Boc]⁺), 275 (56.5, [*M* – 2(*t*-Bu)]⁺), 257 (7.5), 231 (18.0, [*M* – (*t*-Bu) – Boc]⁺), 229 (14.2, [*M* – 2(*t*-Bu) – OEt]⁺), 211 (9.1), 185 (7.5), 156 (4.8), 139 (20.3), 128 (17.5), 101 (7.9), 84 (7.5), 74 (4.0), 56 (5.1). Anal. calc. for C₁₉H₃₄N₂O₆ (386.49): C 59.05, H 8.87, N 7.25; found: C 59.23, H 8.65, N 7.16.

O-Ethylcoprine (=N⁵-(1'-Ethoxycyclopropyl)-L-glutamine; **3**). A suspension of **17** (36 mg, 0.156 mmol) in aq. 1.2M HCl (0.5 ml) was heated to 40°. After 30 min (TLC (cellulose, BuOH/acetone/H₂O/Et₂NH 10:5:5:2): no **17** at R_f 0.97), H₂O (2 ml) was added and the mixture evaporated. The solid residue was purified by cation-exchange chromatography (Amberlite CG-120-II, H⁺ form, aq. 0.3N NH₃): 16.8 mg (78.5%) of **3**. The colorless crystals were recrystallized from H₂O/EtOH. TLC (cellulose, BuOH/acetone/H₂O/Et₂NH 10:5:5:2): R_f 0.55, pure. M.p. 179–181° ([5]: 183–184°). [α]_D²⁰ = +3.3 (*c* = 4.4, H₂O; [5]: [α]_D²⁰ = +5.2 (*c* = 7.8, H₂O)); [α]_D²⁰ = +4.8 (*c* = 4.4, H₂O). IR (KBr): 3420s (br., NH), 3010w, 2990m (CH), 1665s (br., CO, NCO I), 1580m, 1525s (br., NCO II), 1450m, 1410s, 1250m, 1185m, 1020m, 970m, 810m. ¹H-NMR (300 MHz, D₂O): 0.88–0.97 (*m*, 2H, CH₂(2'), CH₂(3')); 1.03–1.22 (*m*, 2H, CH₂(2'), CH₂(3')); 1.09 (*t*, *J* = 7.1, CH₃CH₂O); 1.99–2.62 (*m*, CH₂(3), CH₂(4)); 3.65 (*q*, *J* = 7.1, CH₃CH₂O); 3.77 (*m*, H–C(2)). FAB-MS (glycerine): 447 (3.68), 405 (2.75), 275 (1.10), 261 (1.13), 259 (2.34), 249 (1.48), 245 (38.30), 233 (0.98), 231 (3.28, [*M* + H]⁺), 217 (2.23), 203 (100, [*M* – C₂H₄]⁺), 186 (15.66, [*M* – EtO]⁺), 170 (3.92), 168 (8.92), 159 (3.75), 157 (9.70), 147 (14.1), 139 (17.05), 130 (18.97), 115 (10.10), 97 (12.02), 84 (39.65), 74 (27.37), 56 (70.38, C₂H₆N), 43 (30.13). Anal. calc. for C₁₀H₁₈N₂O₄ (230.26): C 52.16, H 7.88, N 12.17; found: C 52.30, H 7.65, N 12.28.

Coprine (=N⁵-(1'-Hydroxycyclopropyl)-L-glutamine; **1**). A suspension of **17** (242 mg, 0.627 mmol) in aq. 2M HCl (5 ml) was heated to 40°. After stirring for 1 h, a mixture of **3** (R_f 0.55) and **1** (R_f 0.33) was present according to TLC (cellulose, BuOH/acetone/H₂O/Et₂NH 10:5:5:2). The mixture was heated to 60°, and after 1 h stirring (TLC: **1** at R_f 0.33 and traces of L-glutamic acid at R_f 0.15), H₂O (10 ml) was added and the mixture evaporated. The solid residue was purified by cation-exchange (Amberlite CG-120-II (H⁺ form, aq. 0.3N NH₃) and anion-exchange chromatography (Amberlite CG-400-II, AcO[–] form, H₂O): 92.4 mg (73%) of **1**. The colorless crystals were recrystallized from H₂O/EtOH. TLC: pure **1**. M.p. 194–197° ([5]: 197–199°). [α]_D²⁰ = +5.9 (*c* = 2.8, H₂O; [5]: [α]_D²⁰ = +7.6 (*c* = 4.1, H₂O)); [α]_D²⁰ = +8.2 (*c* = 2.8, H₂O). IR (KBr): 3380s (br., NH), 3020w, 2995m (CH), 1680s (br., CO, NCO I), 1590m, 1540s (br., NCO II), 1455m, 1420s, 1260m, 1195m, 1130m, 1040m, 980m, 825m. ¹H-NMR (400 MHz, D₂O): 0.92–1.10 (*m*, 2H, CH₂(2'), CH₂(3')); 1.11–1.19 (*m*, 2H, CH₂(2'), CH₂(3')); 2.04 (*s*, traces of AcO[–]); 2.05–2.17 (*m*, CH₂(3)); 2.40 (*t*, *J* = 7.2, CH₂(4)); 3.77 (*t*, *J* = 6.6, H–C(2)). ¹³C-NMR (101 MHz, D₂O): 22.36, 25.70 (C(2'), C(3')); 30.90 (C(3)); 48.61 (C(4)); 60.82 (C(2)); 66.33 (C(1')); 179.45, 181.15 (C(5), C(1)). FAB-MS (glycerine): 405 (0.63), 317 (1.23), 262 (1.25), 244 (1.19), 240 (5.00), 225 (11.62), 203 (100, [*M* + H]⁺), 186 (13.10, [*M* – OH]⁺), 166 (4.30), 157 (4.17), 148 (44.34, [Gln + H]⁺), 130 (17.53), 110 (50.58), 100 (5.27), 84 (19.79), 74 (52.29), 56 (10.44). Anal. calc. for C₈H₁₄N₂O₄ (202.21): C 47.52, H 6.98, N 13.85; found: C 47.31, H 6.70, N 13.89.

REFERENCES

- [1] G. Bresadola, 'I funghi mangerecci e velenosi', Scotoni, Trient, 1906; I. Fischer, *Svensk Läkartidning* **1945**, *42*, 2513; H. Cléménçon, *Schweiz. Zeitschr. Pilzk.* **1962**, *40*, 170; P. H. List, H. Reith, *Arzneim.-Forsch.* **1960**, *10*, 34.
- [2] J. Hald, E. Jacobsen, V. Larsen, *Acta Pharmacol. Toxicol.* **1948**, *4*, 285; L. Lundwall, F. Baekeland, *J. Nerv. Ment. Disease* **1971**, *153*, 381.
- [3] P. Lindberg, R. Bergman, B. Wickberg, *J. Chem. Soc., Chem. Commun.* **1975**, 946; G. M. Hatfield, J. P. Schaumberg, *Lloydia* **1975**, *38*, 489.
- [4] O. Tottmar, P. Lindberg, *Acta Pharmacol. Toxicol.* **1977**, *40*, 476.
- [5] P. Lindberg, R. Bergman, B. Wickberg, *J. Chem. Soc., Perkin Trans. 1* **1977**, *6*, 684.
- [6] S. Tsukamoto, S. Chiba, T. Sudo, T. Muto, S. Oshida, *Nihon Univ. J. Med.* **1986**, *28*, 359.
- [7] T. Kienzler, P. Strazewski, Ch. Tamm, *Synlett* **1991**, *10*, 737.
- [8] W. J. M. van Tilborg, S. E. Schaafsma, H. Steinberg, T. J. de Boer, *Synth. Commun.* **1973**, *3*, 189; P. Lindberg, Thesis, University of Lund, 1977; W. J. M. van Tilborg, Thesis, University of Amsterdam, 1979.
- [9] P. Strazewski, Ch. Tamm, *Synthesis* **1987**, 298.
- [10] D. H. R. Barton, D. Bridon, S. Z. Zard, *J. Chem. Soc., Chem. Commun.* **1985**, 1066.
- [11] T. Kienzler, Dissertation, Universität Basel, 1991.
- [12] H. Hofer, M. Moest, *Liebigs Ann. Chem.* **1902**, *323*, 285.
- [13] R. P. Linstead, B. R. Shephard, B. C. L. Weedon, *J. Chem. Soc.* **1951**, 2854; H. G. Thomas, S. Kessel, *Chem. Ber.* **1988**, *121*, 1575; D. Seebach, R. Charczuk, C. Gerber, P. Renaud, *Helv. Chim. Acta* **1989**, *72*, 401.
- [14] T. H. Koch, R. J. Sluski, *Tetrahedron Lett.* **1970**, 2391.
- [15] M. Bodanszky, in 'Principles of Peptide Synthesis', Springer Verlag, Berlin, 1984, p. 98.
- [16] J. R. Vaughan, R. L. Osato, *J. Am. Chem. Soc.* **1951**, *73*, 5553.
- [17] W. König, R. Geiger, *Chem. Ber.* **1970**, *103*, 788.
- [18] B. Castro, J. R. Dormoy, G. Evin, C. Selve, *Tetrahedron Lett.* **1975**, 1219.
- [19] A. K. Saha, P. Schultz, H. Rapoport, *J. Am. Chem. Soc.* **1989**, *111*, 4856.
- [20] D. H. R. Barton, Y. Hervé, P. Potier, J. Thierry, *J. Chem. Soc., Chem. Commun.* **1984**, 1298.