

Fig. 4. 'Shatter cone' with a nearly ideal cone-shaped structure in quartz gangue with little feldspar and muscovite from the locality 'Pos Cota', E of Lago di Tremorgio, at height ca. 1920 m. The cone diameter becomes smaller over a distance of only 20 cm, from about 17 cm (left) to nearly 0 cm (right, in the center of the rock). Note the feldspar ( $4 \times 12$  cm) like part of a mantle ( $\varnothing$  some mm) at the thick end of the cone (below). Dimension of the rock  $20 \times 20 \times 38$  cm long. Photograph by K. PFISTER, Photograph. Institute of ETH Zürich.

cular to each other and kinked. In the plagioclase bent albite twin lamellae (up to  $10^\circ$ ) may also appear and grade into optically individual segregations of  $50 \times 100 \mu\text{m}$  and a kink angle up to  $25^\circ$  against the host mineral, whilst scalariform lamellae perpendicular to the twin lamellae resemble planar features. The same optically globular segregations as in the quartz were also observed in plagioclase, but more rarely. Sometimes the small spheres around the vesicles are strung like a string of beads along planes parallel to fine fractures. In other minerals, carbonates and other silicates, no indisputable indications for shock metamorphism have been found in the samples so far collected.

In addition to detailed field investigations<sup>5</sup>, various physical tests for shock metamorphism<sup>6-10</sup> will be made in the near future. In particular a search will be made for the very high pressure minerals coesite<sup>11,12</sup> and/or stishovite<sup>13,14</sup> in the quartzose rocks of that region<sup>15</sup>.

<sup>5</sup> K. BÄCHTIGER, Schweiz. miner. petrogr. Mitt., in press (1976).

<sup>6</sup> B. M. FRENCH and N. M. SHORT, *Shock Metamorphism of Natural Materials* (Mono Book Corporation, Baltimore 1968), p. 644.

<sup>7</sup> D. J. McDOUGALL, *Application of Thermoluminescence to Geological Problems*. Proc. NATO Adv. Research Institute (Academic Press, London and New York 1968), p. 678.

<sup>8</sup> K. BÄCHTIGER, F. HOFMANN and F. LAVES, 9e Congrès Internationale de Sédimentologie (SEDIM 75), Nice 5-14 Juillet 1975, thème 10, p. 1.

<sup>9</sup> K. BÄCHTIGER and F. HOFMANN, *Eclog. geol. Helv.* **69**, 204 (1976).

<sup>10</sup> F. HOFMANN and K. BÄCHTIGER, *Eclog. geol. Helv.* **69**, 177 (1976).

<sup>11</sup> E. C. T. CHAO, E. M. SHOEMAKER, and B. M. MADSEN, *Science* **132**, 220 (1960).

<sup>12</sup> E. M. SHOEMAKER and E. C. T. CHAO, *J. geophys. Res.* **66**, 3371 (1961).

<sup>13</sup> E. C. T. CHAO, J. J. FAHEY, J. LITTLER and D. J. MILTON, *J. geophys. Res.* **67**, 419 (1962).

<sup>14</sup> E. C. T. CHAO and J. LITTLER, *Geol. Soc. Am. Abstr.* **1962**, 27 A (1963).

<sup>15</sup> F. BIANCONI, *Beitr. geol. Karte Schweiz* **142**, 238 (1971).

## Synthesis of Eel-Calcitonin and [Asu<sup>1,7</sup>]-Eel-Calcitonin: Contribution of the Disulfide Bond to the Hormonal Activity

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**Summary.** Eel-calcitonin and its [Asu<sup>1,7</sup>]-analog, deamino-dicarba-analog, were synthesized by the conventional solution method. The natural-type product showed 4300 MRC U/mg in the hypocalcemic potency which was comparable to that of the natural hormone isolated from eel. Hormonal activity of the Asu-analog was also as high as 3400 MRC U/mg.

In 1974, OTANI et al.<sup>2</sup> had succeeded in isolating a hypocalcemic factor from the pericardium and esophagus of eel, and the amino acid sequence was elucidated by NODA and NARITA<sup>3</sup> as shown below.

Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asp-Val-Gly-Ala-Gly-Thr-Pro-NH<sub>2</sub>

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<sup>2</sup> M. OTANI, T. NODA, H. YAMAUCHI, S. WATANABE, T. MATSUDA, H. ORIMO and K. NARITA, *Proc. 5th Parathyroid Conference*, Oxford (Eds. R. V. TALMAGE, M. OWEN and J. A. PARSONS; Excerpta Medica 1974), p. 111. — M. OTANI, H. YAMAUCHI, T. MEGURO, S. KITAZAWA, S. WATANABE and H. ORIMO, *J. Biochem.*, Tokyo **79**, 345 (1976).

<sup>3</sup> T. NODA and K. NARITA, *J. Biochem.*, Tokyo **79**, 353 (1976).

The structure is quite similar to that of salmon calcitonin I<sup>4</sup>: only 3 amino acid residues, Asn<sup>26</sup>, Thr<sup>27</sup>, and Ser<sup>29</sup>, in salmon calcitonin are replaced by Asp, Val and Ala, respectively, in eel. A highly purified sample of the eel calcitonin (E-CT) shows hypocalcemic potencies of 3500 to 5000 MRC units per mg<sup>5</sup>, which are in the same range of the reported values of salmon calcitonin I; 5000 MRC units<sup>5</sup> and 2300–3200 MRC units/mg<sup>6</sup> for the isolated samples, and 3500 MRC units/mg<sup>7</sup> for a synthetic sample. The present report deals with the synthesis of a peptide with the above structure and of an analog, in which the *N*-terminal amino group and the SS-bond of the natural peptide were replaced by a hydrogen atom and by an ethylene linkage, respectively. The concept of preparing this kind of analog was originally introduced by Jösr and RUDINGER<sup>8</sup> and by a group of a present author (S.S.)<sup>9</sup>, independently, in the field of neurohypophyseal hormones.

Synthesis of the natural-E-CT was carried out by taking a route indicated in Figures 1 and 2<sup>10</sup>. All the functional groups in the amino acid residues were protected by groups which are used commonly in the solid

phase synthesis. Each fragment was purified by the conventional procedures, and applied to the next step after confirming the homogeneity by thin layer chromatography and by amino acid analysis. A part of the Fragment I was treated with anhydrous HF<sup>11</sup> followed by column chromatography on CM-cellulose to obtain a free peptide, which was completely digestible by aminopeptidase M. The final protected peptide was treated with anhydrous HF at –5 °C for 1 h in the presence of anisole. The deprotected material was purified twice by column chromatography on CM-cellulose using a linear gradient concentration of an ammonium acetate buffer (0.01 to 0.15 *M*) at pH 4.5 followed by Sephadex LH-20 chromatography using 0.1 *M* acetic acid as an eluent. The final product showed a single spot of R<sub>f</sub> 0.69 on cellulose thin layer chromatography with a solvent system of *n*-BuOH: AcOH: pyridine: H<sub>2</sub>O (15:3:10:12 v/v/v/v); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –86° ± 3° (c 0.22, 0.1 *M* HCOOH); the reported value<sup>2</sup> for the

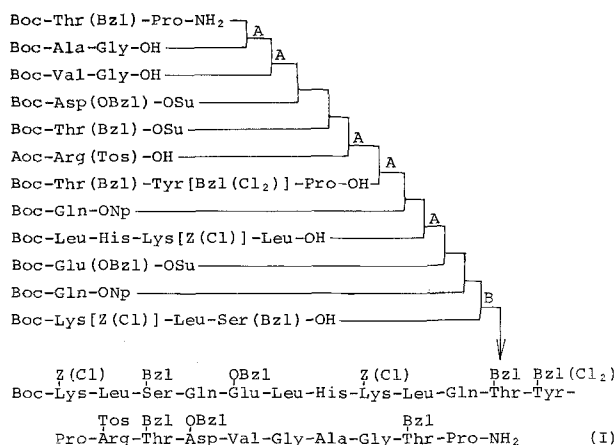


Fig. 1. Synthesis of fragment I (11–32)<sup>10</sup>.

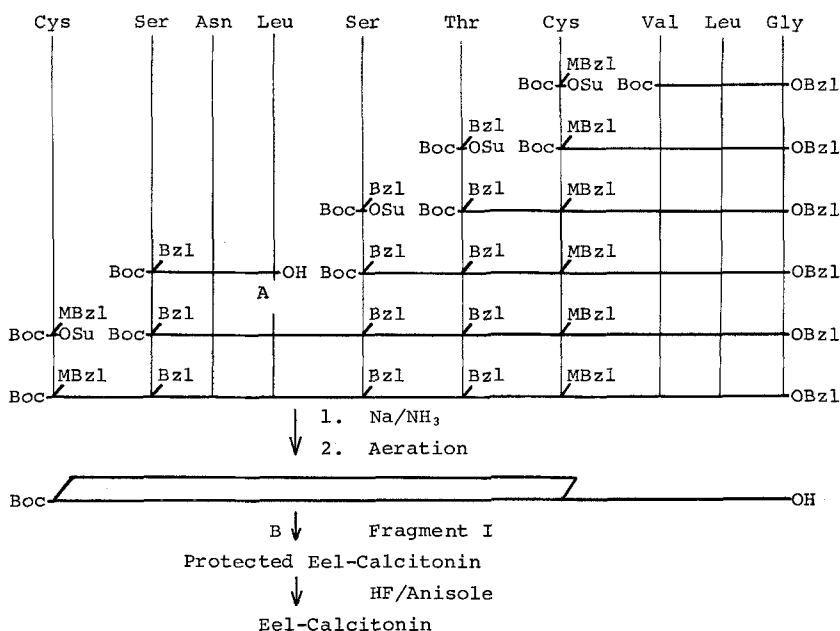


Fig. 2. Synthesis of eel-calcitonin<sup>10</sup>.

<sup>4</sup> H. D. NIALI, H. T. KEUTMANN, D. H. COPP and J. T. POTTS, JR., *Proc. natn. Acad. Sci., USA* **64**, 771 (1969).

<sup>5</sup> R. K. O'DOR, C. O. PARKES and D. H. COPP, *Can. J. Biochem.* **47**, 823 (1969).

<sup>6</sup> H. T. KEUTMANN, J. A. PARSONS, J. T. POTTS, JR. and R. J. SCHLUETER, *J. biol. Chem.* **245**, 1491 (1970).

<sup>7</sup> St. GUTTMANN, J. PLESS, R. L. HUGUENIN, Ed. SANDRIN, H. BOSSERT and K. ZEHNDER, *Helv. chim. Acta* **52**, 1789 (1969).

<sup>8</sup> K. JÖSR and J. RUDINGER, *Collect. Czech. chem. Commun.* **32**, 1229 (1967).

<sup>9</sup> S. SAKAKIBARA and S. HASE, *Bull. chem. Soc. Japan* **41**, 2816 (1968). – A. KOBAYASHI, S. HASE, R. KIYOI and S. SAKAKIBARA, *Bull. chem. soc. Japan* **42**, 3491 (1969). – S. HASE, T. MORIKAWA and S. SAKAKIBARA, *Experientia* **25**, 1239 (1969).

<sup>10</sup> Amino acids except Gly denote the L-configuration. Abbreviations are in accordance with the recommendations of IUPAC-IUB Commission (*J. biol. Chem.* **247**, 977 (1972)). Z(Cl), 2-Chlorobenzyl-oxycarbonyl; HOSu, *N*-Hydroxysuccinimide; Bzl(Cl<sub>2</sub>), 2,6-Dichlorobenzyl; MBzl, *p*-Methoxybenzyl; TFA, Trifluoroacetic acid; DMF, *N,N*-Dimethylformamide; A, C<sub>6</sub>H<sub>5</sub>-N=C=N-(CH<sub>2</sub>)<sub>3</sub>-N(CH<sub>2</sub>)<sub>2</sub>/1-Hydroxybenzotriazole method; B, DCC/HOSu method. The Boc group in each fragment was removed by TFA.

<sup>11</sup> S. SAKAKIBARA and Y. SHIMONISHI, *Bull. chem. Soc. Japan* **38**, 1412 (1965). – S. SAKAKIBARA, Y. SHIMONISHI, Y. KISHIDA, M. OKADA and H. SUGIHARA, *Bull. chem. Soc. Japan* **40**, 2164 (1967).

natural product was  $[\alpha]_D^{25} -88^\circ$  (c 0.1, 0.1 M HCOOH). The ratio of amino acids in an acid hydrolyzate: Cys 2.13(2), Ser 2.86(3), Leu 5.10(5), Thr 3.48(4), Val 2.20(2), Gly 3.30(3), Lys 1.98(2), Glu 3.27(3), His 0.95(1), Tyr 0.90(1), Pro 1.96(2), Arg 1.03(1), Asp 2.07(2), Ala 0.94(1). (Average recovery was 89.9%)

The head part of  $[\text{Asu}^{1,7}]$ -E-CT, which corresponds to the sequence (1–10) of the natural hormone, was synthesized as shown in Figure 3. Almost the same procedure used for the synthesis of  $[\text{Asu}^{1,6}]$ -oxytocin<sup>9</sup> was applied to cyclization of the fragment (2–7). The cyclized product was further coupled with Val-Leu-Gly to obtain a protected nonapeptide with a free carboxyl group at the C-terminus. This material was converted to the *N*-hydroxy-succinimide ester, which was then coupled with the fragment I in DMF at 30°C. The crude product, fully protected  $[\text{Asu}^{1,7}]$ -E-CT, was treated with anhydrous HF as in the case of the synthesis of the natural hormone, and the liberated free peptide was purified by the use of CM-cellulose column chromatography under the same conditions as above, Sephadex G-25 partition chromatography with a solvent system of *n*-BuOH: Pyridine: 1% AcOH (5:3:11 v/v/v), and gel-filtration with Sephadex LH-20 using 0.1 M AcOH as a solvent, successively. Thus, homogeneous  $[\text{Asu}^{1,7}]$ -E-CT was obtained in an over-all yield of more than 30% through the final coupling and purification procedures: Rf 0.71 on cellulose thin layer chromatography using a mixture of *n*-BuOH: AcOH: Pyridine: H<sub>2</sub>O (15:3:10:12 v/v/v/v) as the solvent;  $[\alpha]_D^{18} -95^\circ \pm 2^\circ$  (c 0.54, 1 M AcOH). The ratio of amino acids in an acid hydrolyzate: Lys 1.92(2), His 0.89(1), Arg 0.95(1), Asp 1.90(2), Thr 3.88(4), Ser 3.00(3), Glu 3.33(3), Pro 2.16(2), Gly 3.00(3), Ala 0.92(1), Val 2.10(2), Leu 4.95(5), Tyr 0.92(1), Asu 0.99(1), (Average recovery 90.1%).

The synthetic E-CT showed a rat hypocalcemic activity of 4300 MRC U/mg using a similar procedure to that outlined by KUMAR et al.<sup>12</sup> This potency was reasonably high in comparison with that of the natural hormone as described above. The synthetic  $[\text{Asu}^{1,7}]$ -analog showed a specific activity of 3400 MRC U/mg under the same bioassay system. These findings indicate clearly that the disulfide bond in calcitonin is essential not for the biological activity but for maintenance of the specific conformation by forming an intramolecular bridge, since an open-chain analog of human CT is known to be inactive<sup>13</sup>. During the course of the final purification of the syn-

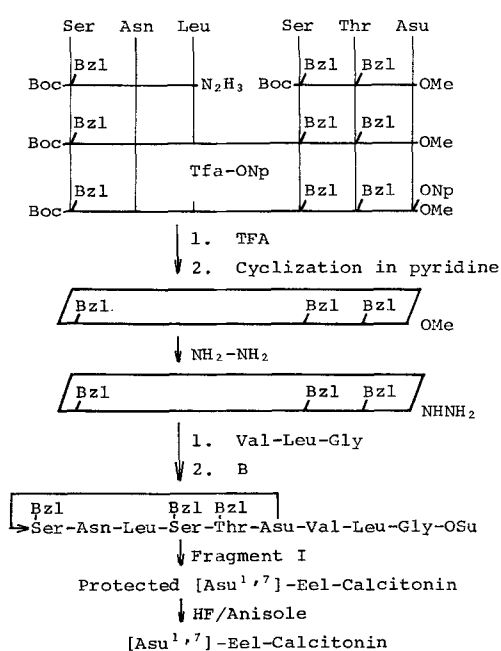


Fig. 3. Synthesis of  $[\text{Asu}^{1,7}]$ -eel-calcitonin<sup>10</sup>.

thetic natural-type E-CT, we were troubled seriously by an unexpected loss of the biological activity; thus, we could not get a reproducible yield of this compound in different runs. In the case of  $[\text{Asu}^{1,7}]$ -analog synthesis, no difficulty was encountered during purification in keeping the biological activity. These facts suggest that the instability of synthetic calcitonin should mainly be attributed to the presence of a disulfide bond, and that, if the Asu-analog were proved not toxic, the use of such an analog must be much more advantageous in developing a hypocalcemic drug because of its ease of manufacturing, enough potency and higher stability during storage.

<sup>12</sup> M. A. KUMAR, E. SLACK, A. EDWARDS, H. A. SOLIMAN, A. BAGHDANTZ, G. V. FOSTER and I. MACINTYRE, J. Endocr. 33, 469 (1965).

<sup>13</sup> W. RITTEL, R. MAIER, M. BRUGGER, B. KAMBER, B. RINKER and P. SIEBER, Experientia 32, 246 (1976).

## A Modified Dipeptide from the Alga *Cystoseira corniculata* Hauck

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**Summary.** A modified L-Phe-L-Phe dipeptide has been isolated from the alga *Cystoseira corniculata*.

Although numerous compounds have been isolated from algae in recent years they include, so far as we are aware, only two simple peptides<sup>2,3</sup>. We have now obtained the modified phenylalanine dipeptide 1 by chloroform extraction of *Cystoseira corniculata*<sup>4</sup> collected at Marmaris, Turkey.

Extraction of the dried alga (550 g) gave a thick green oil (2 g) which, after column and repeated thin layer chromatography, afforded the dipeptide (57 mg) as needles, m.p. 185–186° (from chloroform-petrol), C<sub>27</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub>

(M<sup>+</sup>, 444.2048),  $[\alpha]_D = -74.0^\circ$  (c, 0.98; CHCl<sub>3</sub>);  $\lambda_{max}$  (MeOH) 214, 227sh (log  $\epsilon$  4.18, 4.07); CD,  $\lambda_{max}$  (MeOH) 212 ( $\Delta\epsilon$  -1.27), 227 nm (-4.74);  $\nu_{max}$  (KBr) 3320 (NH), 1728 (ester CO), 1662 and 1634 cm<sup>-1</sup> (-NHCO-). The <sup>1</sup>H NMR-spectrum (CDCl<sub>3</sub>) showed signals for an acetate methyl group ( $\delta$  2.00, s), 3 methylene (2.74d, 3.15 m, and 3.88 m), 2 methine (4.34 m and 4.83 q), 2 NH (6.26 d and 6.88 d, exchanged with D<sub>2</sub>O), and 15 aromatic protons. Decoupling experiments showed that the methine proton at  $\delta$  4.83 was coupled to the methylene group at  $\delta$  3.15