Biological apatites. The expanded *a*-axis of the human enamel apatite has been explained as being due to the partial substitution of CO_3-for-OH in the apatite structure[5]. However, it has been demonstrated from these studies that all apatites precipitated from aqueous systems demonstrate longer *a*-axes than those heated to high temperatures or those of the natural mineral apatites. Furthermore, since the CO_3-for-OH substitution is accomplished only with the exclusion of water, it is difficult to conceive of such substitution in biological apatites. The IR-absorption spectra of biological apatites are similar to those of precipitated apatites in which CO_3-for-PO_4 substitution takes place (Figure 2). The V_2 CO_3 doublet at 871 and 878 cm^{-1} in the spectra of biological apatites have been interpreted to indicate lattice carbonate (substituting for OH groups) and adsorbed carbonate[5]. This doublet is also observed in the spectra of carbonate-containing precipitated apatites[11]. The similarities of the characteristics of the carbonate bands in the spectra of precipitated and biological apatites suggest that the carbonates in these apatites experience the same environment[11–14].

Table III. Frequency assignments of the absorption bands of OH, CO_3, PO_4 in the spectra of carbonate-containing synthetic and biological apatites

Vibrating group	Modes	Synthetic A[a]	Synthetic B[b]	H. Enamel
OH		–	3580 cm^{-1}	3575 cm^{-1}
		–	1624	1630
PO_4	V_1	950	957	955
	V_3	1045	1090	1080
		1025	1040	1035
	V_4	602	602	602
		572	562	562
	comb.	455	470	472
CO_3	V_2	877	870	870
			877	878
	V_3	1460	1410	1410
		1525	1450	1460
		1550	1540	1545

[a] Prepared at high temperatures (1000 °C). CO_3-for-OH substitution.
[b] Precipitated at 100 °C. CO_3-for-PO_4 substitution.

Zusammenfassung. Um die Art des Karbonateinbaues in die Apatitstruktur zu klären, wurden zwei Typen von synthetischen Karbonatapatiten untersucht: solche, die sich in wässrigen Medien bildeten, und andere, die bei hohen Temperaturen und unter Ausschluss von Wasser entstanden.

R. Z. LeGeros, O. R. Trautz, E. Klein and J. P. LeGeros

New York University, College of Dentistry, New York (N.Y. 10010) and The Cooper Union, New York (N.Y., USA), 12 August 1968.

[11] R. Z. LeGeros, J. P. LeGeros, O. R. Trautz and E. Klein, Proc. Soc. appl. Spec. *7*, in press (1968).
[12] R. Z. LeGeros, O. R. Trautz, J. P. LeGeros and E. Klein, Bull. soc. Chim. Fr., No. special 1712 (1968).
[13] We acknowledge the technical assistance of C. S. Strom and G. M. Domingo. This program is supported by U.S.P.H.S. Grant No. DE-00159.
[14] We are grateful to Dr. J. C. Elliott of the University of London for giving us the apatites prepared at high temperatures.

Synthesis of Phyllocaerulein

We report the synthesis of a nonapeptide of the formula Pyr-Glu-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2 according to the following scheme. The product was found to be identical with natural phyllocaerulein[1–3].

Condensation of Boc-Thr[5] with Z-NH-NH_2 via the mixed anhydride in THF afforded the protected hydrazide I (97% yield) which was directly treated with AcCl in AcOH/HCl 6N to give $^+H_2$-Thr(Ac)-NH-NH-Z·Cl^- (II) (68% yield; m.p. 125–126°; $[\alpha]_D^{22}$ + 10°, *c* = 1, DMF; $E_{1.2}$ = 0.94 Glu. *Anal.* Calcd. for $C_{14}H_{19}N_3O_5$·HCl: C 48.6; H 5.8; N 12.1; Cl 10.2. Found C 48.2; H 6.2; N 12.3; Cl 9.8).

Boc-Tyr[5] was condensed, via the mixed anhydride, with II in THF/DMF in the presence of 1 equivalent of MM to give Boc-Tyr-Thr(Ac)-NH-NH-Z (III) (61% yield; m.p. 133–134°; $[\alpha]_D^{21}$ – 1.6°, *c* = 1, DMF. *Anal.* Calcd. for $C_{28}H_{36}N_4O_9$: C 58.7; H 6.3; N 9.8. Found C 58.5; H 6.3; N 9.6).

Treatment of III with HCl/AcOH 1.3 N afforded $^+H_2$-Tyr-Thr(Ac)-NH-NH-Z·Cl^- (IV) (100% yield; m.p. 150–160°; $[\alpha]_D^{21}$ + 29°, *c* = 1, AcOH 95%. *Anal.* Calcd. for $C_{23}H_{28}N_4O_7$·HCl: C 54.3; H 5.8; N 11.0. Found C 54.3; H 5.9; N 10.7) which was condensed, via the mixed anhydride, with Boc-Glu(OBzl)[6] in THF/DMF in the presence of 1 equivalent of MM to give Boc-Glu(OBzl)-Tyr-Thr(Ac)-NH-NH-Z (V) (85% yield; m.p. 183–184°; $[\alpha]_D^{21}$ – 3.6°, *c* = 1, DMF. *Anal.* Calcd. for $C_{40}H_{49}N_5O_{12}$: C 60.7; H 6.2; N 8.8. Found C 60.5; H 6.1; N 8.8).

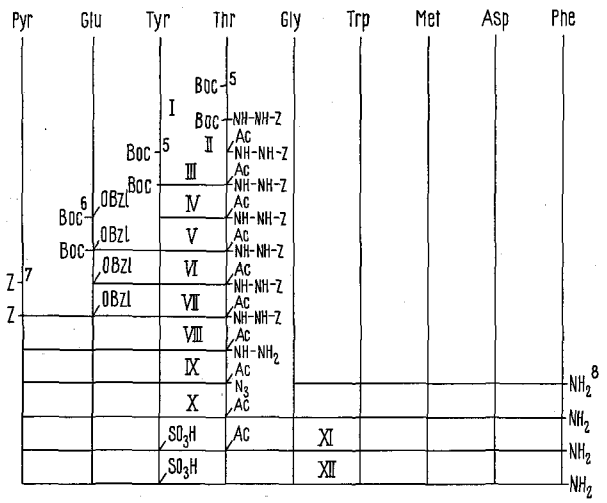
In the same way, treatment of V with HCl/AcOH 1.3 N gave $^+H_2$-Glu(OBzl)-Tyr-Thr(Ac)-NH-NH-Z·Cl^- (VI) (86% yield; m.p. 182°; $[\alpha]_D^{21}$ + 28°, *c* = 1, AcOH 95%; $E_{1.2}$ = 0.56 Glu. *Anal.* Calcd. for $C_{35}H_{41}N_5O_{10}$·HCl: C 57.7; H 5.8; N 9.6. Found C 57.6; H 6.0; N 9.3) which on condensation with Z-Pyr[7], via the mixed anhydride, in THF/DMF in the presence of 1 equivalent of MM, afforded the protected tetrapeptide Z-Pyr-Glu(OBzl)-Tyr-Thr(Ac)-NH-NH-Z (VII) (70% yield; m.p. 222–224°;

[1] A. Anastasi and V. Erspamer, 3rd Symposium of the European Pancreatic Club, Prague 2–4 July 1968.
[2] A. Anastasi, Experientia *25*, 8 (1969).
[3] All the amino acids have the L-configuration. The following abbreviation are used throughout this paper[4]: Z, benzyloxycarbonyl; Boc, *t*-butyloxycarbonyl; OBzl, benzyl ester; Ac, acetyl; *n*-Bu, *n*-butyl; Et, Ethyl; MM, N-methylmorfoline; THF, tetrahydrofuran; DMF, dimethylformamide; DMSO, dimethylsulfoxide; Cys(SO_3H), cysteic acid.

$[\alpha]_D^{22}$ −9.2°, $c = 1$, DMF. *Anal.* Calcd. for $C_{48}H_{52}N_6O_{14}$: C 61.5; H 5.6; N 9.0. Found C 61.8; H 5.8; N 8.9).

Hydrogenation of VII in DMF in the presence of Pd/C 10% gave the key intermediate Pyr-Glu-Tyr-Thr(Ac)-$NH-NH_2$ (VIII) (99% yield; m.p. 193–194°; $[\alpha]_D^{22}$ +2.8°, $c = 0.6$, DMF; $E_{1.2} = 0.48$ Glu) which was converted into the azide IX by treatment at −25° with HCl/THF 2N and *t*-butyl nitrite, and condensed with the pentapeptide $^+H_2$-Gly-Trp-Met-Asp-Phe-$NH_2 \cdot Cl^-$ [8] in DMF at −12° for 4 days to give the nonapeptide Pyr-Glu-Tyr-Thr(Ac)-Gly-Trp-Met-Asp-Phe-NH_2 (X) (83% yield; m.p. 208 to 210°; $[\alpha]_D^{22}$ −15°, $c = 1$, DMF; $E_{5.8} = 0.28$ Glu. *Anal.* Calcd. for $C_{56}H_{69}N_{11}O_{17}S \cdot {}^1/_2 H_2O$: C 55.6; H 5.8; N 12.7. Found C 55.7; H 5.9; N 12.3).

The product was next treated in pyridine-DMF with 7 equivalents of SO_3/pyridine complex for 5 h. After evaporation of the solvent in vacuo and dissolution of the residue in the bottom layer (A) of the system *n*-BuOH-EtOH-H_2O (5:1:8), NaOH was added to pH 3.2 and the solution was extracted with the top layer (B) of the same system. Evaporation of the solvent left a crude residue (XI) which was dissolved in A and made basic with NaOH to pH 11. After 3 h HCl was added to pH 3.2 and the solution extracted with B. Evaporation of the solvent left a residue of crude peptide which was purified by elution from DEAE – Sephadex (OH^-) with 1 *M* $(NH_4)_2CO_3$ buffer and deionized on Amberlite CG-50 (H^+). The nonapeptide Pyr-Glu-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2 (XII) so obtained (48% yield; $E_{1.9} = 0.57$ Cys(SO_3H); $E_{5.8} = 0.50$ Glu; 0.39 Cys(SO_3H)) was found to be homogeneous and showed the same electrophoretic and chromatographic properties, the same behaviour towards chymotrypsin and subtilisin, and the same degradation pattern and biological properties as natural phyllocaerulein, thus confirming the formula previously deduced from degradation experiments.



Synthesis of phyllocaerulein.

Riassunto. Viene riportata la sintesi della piroglutamil-glutamil-tirosil(0-solfato)-treonil-glicil-triptofanil-metionil-aspartil-fenilalanilamide, un nonapeptide identico per proprietà chimiche, fisiche e biologiche alla Phyllocaeruleina.

L. BERNARDI, G. BOSISIO,
R. DE CASTIGLIONE and O. GOFFREDO

Istituto Ricerche Farmitalia,
Milano (Italy), 30 September 1968.

[4] IUPAC-IUB Commission on Biochemical Nomenclature, Biochemistry *5*, 2485 (1966).
[5] E. SCHNABEL, Justus Liebigs Annln Chem. *702*, 188 (1967).
[6] J. C. ANDERSON, G. W. KENNER, J. K. LEOD and R. C. SHEPPARD, Tetrahedron, suppl. *8*, 39 (1966).
[7] H. GIBIAN and E. KLIEGER, Justus Liebigs Annln Chem. *640*, 145 (1961).
[8] L. BERNARDI, G. BOSISIO, R. DE CASTIGLIONE and O. GOFFREDO, Experientia *23*, 700 (1967).

The Enzymatic Degradation of Phyllocaerulein and Analogs

Phyllocaerulein, a nonapeptide with a structure very similar to, and activity spectrum identical with that of caerulein[1,2], has been isolated from the skin of a South American amphibian *Phyllomedusa sauvagi*[3]. The structure of phyllocaerulein has been proved by synthesis[4]. As shown in Figure 1, it differs from that of caerulein only in the lack of glutamine and in the substitution of 1 aspartyl with a glutamyl residue.

Caerulein Pyr-Gln-Asp-Tyr(S)-Thr-Gly-Trp-Met-Asp-Phe-NH_2

Phyllocaerulein Pyr-Glu-Tyr(S)-Thr-Gly-Trp-Met-Asp-Phe-NH_2

The sequential analysis of phyllocaerulein has been based, like that of caerulein, on the enzymatic degradation with chymotrypsin and subtilisin. The purpose of this communication is to describe briefly a discrepancy that has been observed in the behaviour of the 2 structures, otherwise so similar, towards the enzymatic attack.

Chymotrypsin behaved in the same way with both peptides. The hydrolysis occurred at the carboxyl side of tryptophan and 2 fragments were produced. In contrast with caerulein, the N-terminal fragment of phyllocaerulein was found to be free of aspartic acid and this immediately confirmed that phyllocaerulein contained only 1 aspartyl residue.

Subtilisin also broke the tryptophan bond of both peptides, and in addition hydrolyzed a second bond, giving rise to 3 fragments. However, this second point

[1] A. ANASTASI, V. ERSPAMER and E. ENDEAN, Experientia *23*, 699 (1967).
[2] A. ANASTASI, V. ERSPAMER and R. ENDEAN, Archs Biochem. Biophys. *125*, 57 (1968).
[3] A. ANASTASI and V. ERSPAMER, Proc. 3rd Symposium of the European Pancreatic Club, Prague 2–4 July 1968 (Czechoslovac Medical Press, Praha), in press.
[4] L. BERNARDI, G. BOSISIO, R. DE CASTIGLIONE and O. GOFFREDO, Experientia *25*, 7 (1969).