problem should induce qualified experimental studies to elucidate the cocarcinogenic mechanism at the molecular level.

Zusammenfassung. Die Übersicht befasst sich mit der Entdeckungsgeschichte der entzündlich, toxisch und kokarzinogen hochwirksamen Phorbolester aus *Croton tiglium* L., wobei insbesondere die Anreicherungs- und Isolierungsversuche sowie die Beiträge zur Chemie der Wirkstoffe berücksichtigt werden. Nach ersten Untersuchungen durch PELLETIER und CAVENTOU im Jahre 1818 erbrachten vor allem die Arbeiten der Arbeitskreise um BOEHM sowie FLASCHENTRÄGER in den zwanziger Jahren dieses Jahrhunderts entscheidende Fortschritte in der chemischen Charakterisierung der Giftstoffe, die als Fettsäureester des Phorbols, $C_{20}H_{28}O_6$, erkannt wurden. Nach der Entdeckung der kokarzinogenen Aktivität hielt das Crotonöl Einzug in die experimentelle Krebsforschung und gab Anlass zu intensiviertem Bemühen um die Isolierung der Wirkstoffe, die durch Anwendung von Gegenstromverteilungen schliesslich 1962 erstmals frei von inaktiven Begleitstoffen dargestellt werden konnten. Inzwischen ist die Chemie der Phorbolester weitgehend geklärt und ein Strukturvorschlag für das Phorbol erarbeitet worden. Die Biochemie dieser Substanzen im Hinblick auf ihre Tumorpromotion bei der Karzinogenese ist jedoch bislang völlig ungeklärt.

SPECIALIA

Two Types of Carbonate Substitution in the Apatite Structure

The apatite structure (e.g. $Ca_{10}(PO_4)_6(OH)_2$) allows varied substitutions to take place (e.g. Sr^{2+} or Ba^{2+} for Ca^{2+}; $(SiO_4)^{4-}$ for $(PO_4)^{3-}$; Cl^- or F^- for $(OH)^-$) without a significant alteration in its basic structure. These substitutions in the structure are manifested by changes in the lattice parameters (*a*- and *c*-axes) of the apatite depending on the size of the substituting cation or anion. The incorporation of carbonate into the apatite structure, however, has been the subject of study and speculations for more than 3 decades. On the basis of chemical analysis, CO_3-for-OH substitution was proposed by EITEL[1]. Carbonate-containing mineral apatites demonstrate a contraction of the unit cell[2–4] but biological apatite (e.g. human enamel) demonstrate an expanded *a*-axis, 9.44 Å. Since all naturally occurring apatites have other constituents besides those of Ca, CO_3 and PO_4, it is necessary to study synthetic systems in which the incorporation of only the carbonate could be directly correlated with the physical properties of the apatite. 2 types of carbonate-containing synthetic apatites have been reported in literature: (a) prepared at high temperatures, 900–1000 °C, with exclusion of water[5,6]; and (b) prepared from aqueous systems by direct precipitation[7,8] and by conversion of monetite, $CaHPO_4$, in carbonate solutions at 100 °C[8]. The results from X-ray diffraction and IR-absorption studies are briefly reported in this paper. A more extensive report will be published elsewhere.

X-ray diffraction analysis. Figure 1 shows diffraction patterns of the 2 types of carbonate-containing synthetic apatites: (A) and (B) are apatites prepared at 1000 °C before and after passing dry CO_2 over them; (C) and (D) are patterns of apatites precipitated from phosphate solutions at 100 °C without and with added carbonate, respectively. The expansion of the *a*-axis in the former case is determined from the shifting of the (300) reflection to lower angles (2θ), and the slight contraction of the *c*-axis from the shifting of the (002) reflection to higher angles. In the precipitated apatites, carbonate incorporation shifts the (300) reflection to higher angles, indicating a contraction of the *a*-axis, and the (002) reflection to lower angles, indicating a slight expansion of the *c*-axis. Since the planar CO_3 group is larger than the (OH) group, an expansion of the unit cell would be expected to accompany a CO_3-for-OH substitution in the apatite (Table I). On the other hand, the planar carbonate is smaller than the tetrahedral PO_4 group, and a contraction of the unit-cell would be expected to accompany a CO_3-for-PO_4 substitution (Table II). It is also of interest to note that the *a*-axes of the precipitated carbonate-free apatites as precipitated, are slightly longer (i.e. by 0.014–0.022 Å) than those of apatites which have been heated at high temperature or those of the mineral OH-apatite from Holly Springs, Ga. (i.e. 9.42_2 Å). This slight expansion of the *a*-axis observed in the precipitated apatites could be due to the presence of some HPO_4^- or entrapped water[4].

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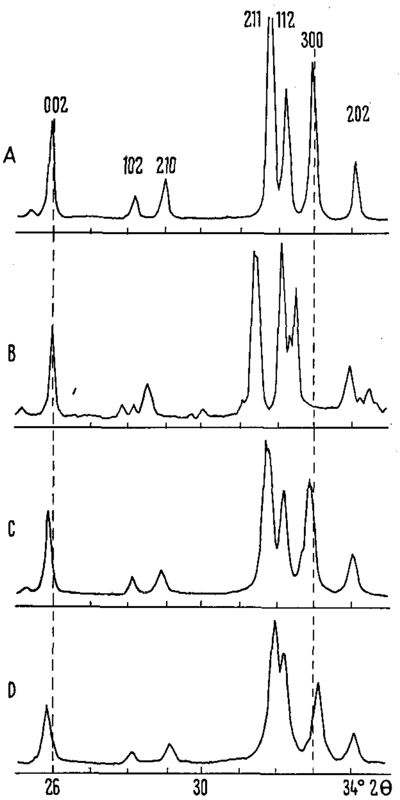

Fig. 1. X-ray diffraction patterns of the 2 types of carbonate-containing synthetic apatites. (A) OH-apatite treated at high temperature ($a = 9.42_2$ Å; $c = 6.88$ Å; weight-% $CO_3 = 0.0$). (B) Prepared by passing dry CO_2 over (A) at 1000 °C ($a = 9.57$ Å; $c = 6.85_1$ Å; weight-% $CO_3 = 5.83$). (C) OH-apatite precipitated at 100 °C ($a = 9.43_6$ Å; $c = 6.88_1$ Å; weight % $CO_3 = 0.5$). (D) Carbonate-containing OH-apatite precipitated (at 100 °C). ($a = 9.40_6$; $c = 6.89_7$ Å; weight-% $CO_3 = 5.80$). Samples (A) and (B) were prepared and given to us by Dr. J. C. ELLIOTT, University of London.

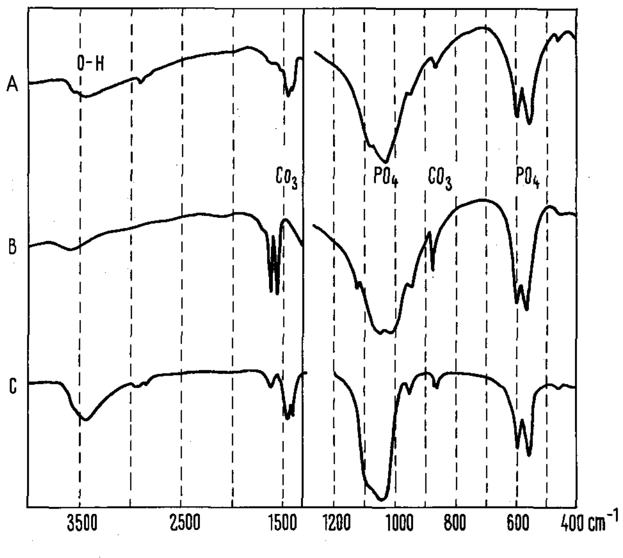

Fig. 2. IR-absorption spectra of carbonate-containing apatites. (A) Biological apatite (human enamel). (Carbonate content 2.7 weight-% CO_3.) (B) Carbonate-apatite prepared at 1000 °C (Carbonate content 5.83 weight-% CO_3). (C) Carbonate-OH-apatite precipitated at 100 °C (Carbonate-content 3.5 weight-% CO_3).

IR-absorption analysis. The difference between the 2 types of synthetic carbonate-containing apatites is also reflected in their IR-absorption spectra (Figures 2B and 2C). Table III lists the corresponding frequencies of the vibration bands of the CO_3, PO_4 and OH groups in the 2 types of carbonate apatites. The significant difference is observed in the V_2 vibration of CO_3: it is observed at 877 cm^{-1} in high temperature apatite (Figure 2B) and as a doublet at 871 and 878 cm^{-1} in precipitated apatite (Figure 2C). The CO_3V_3 vibration is degenerate in both spectra but are shifted to higher frequencies in the high temperature apatites. The additional band at 1125 cm^{-1} in the spectrum of high temperature apatite is unassigned.

Table I. Lattice parameters of apatites treated at high temperatures with CO_2 (900–1000 °C): CO_3-for-OH substitution

Type of apatite	Weight-% CO_3	Lattice parameters a-axis (Å)	 c-axis (Å)
Calcium	0.0	9.42	6.88[5,6]
Calcium	3.49	9.518	6.851[6]
Calcium	1.77	9.457	6.878[5]
Calcium	2.31	9.469	6.874[5]
Calcium	3.67	9.492	6.867[5]
Calcium	4.39	9.544	6.859[5]
Barium	0.0	10.15	7.69[9]
Barium	3.0	10.20	7.65[9]
Strontium	0.0	9.76	7.28[10]
Strontium	3.58	9.88	7.24[10]

Table II. Lattice parameters of carbonate-containing precipitated apatites (100 °C): CO_3-for-PO_4 substitution

Type of apatite	Weight-% CO_3	Lattice parameters a-axis (Å)	 c-axis (Å)
Calcium	0.50	9.43_6	6.87_9[8]
Calcium	2.50	9.42_2	6.88_7[8]
Calcium	4.28	9.41_7	6.88_9[8]
Calcium	8.96	9.38_7	6.89_5[8]
Calcium	15.10	9.35_3	6.90_6[8]
Calcium	20.53	9.31_1	6.92_1[8]
Calcium	22.22	9.30_4	6.93_2[8]
Calcium:	0.51	9.47_7	6.92_2[8]
strontium (9:1)	15.84	9.38_6	6.95_3[8]
Calcium:	0.40	9.48_9	6.89_5[8]
barium (9:1)	10.51	9.42_7	6.91_3[8]
	16.73	9.38_0	6.92_5[8]
Strontium	0.20	9.76_8	7.27_4[8]
Strontium	27.50[a]	9.70_3	7.25_9[8]
Lead	0.21	9.76_8	7.19_7[b]
Lead	8.20	9.72_1	7.20_2[8]
Calcium,	0.30	9.45_5	6.89_3[8]
lead (9:1)	14.68	9.34_7	6.91_9[8]

[a] Contains also $SrCO_3$. [b] This work.

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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.

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[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 $C_{14}H_{19}N_3O_5 \cdot HCl$: 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 $C_{23}H_{28}N_4O_7 \cdot HCl$: 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 $C_{35}H_{41}N_5O_{10} \cdot HCl$: 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°;

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[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.