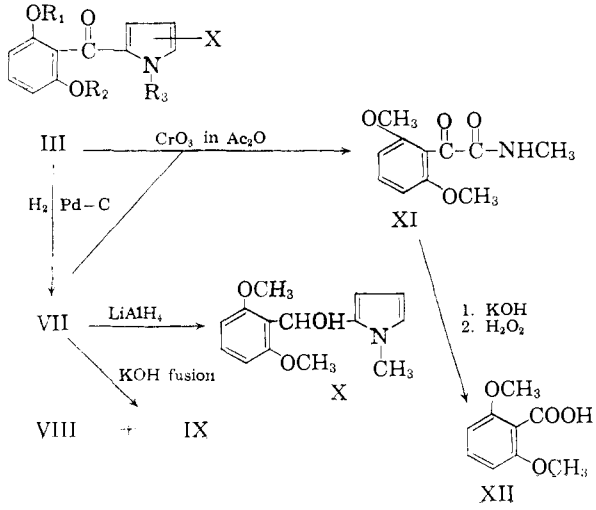
optically inactive. Acetylation of I with acetic anhydride in benzene gave an *O,O'*-diacetate (II) (m.p. 208°; $\lambda_{max.}^{Nujol}$ 3.09 μ (NH), 5.68, 5.77(ester C=O), 6.10 (conj. C=O); found: C, 50.53; H, 3.07; N, 3.83; Cl, 20.00; $COCH_3$,23.81). Methylation of I either with diazomethane or with dimethyl sulfate gave a trimethyl compound (III) (m.p. 135-136°; $\lambda_{max.}^{Nujol}$ 6.09 μ (conj. C=O), no OH-band, no NH-band; found: C, 53.28; H, 4.15; N, 4.63; Cl, 22.51; OCH_3, 19.49; N-CH_3, 4.44). On catalytic reduction of II in the presence of palladium-charcoal a dechloro compound (IV) (m.p. 110°; $\lambda_{max.}^{Nujol}$ 3.07 μ (NH), 5.70(ester C=O), 6.28(conj. C=O); found: C, 62.55; H, 4.63; N, 4.90) was obtained, which, on hydrolysis with potassium hydrogen carbonate, gave dechloropyoluteorin (V) (m.p. 142-143°, $\lambda_{max.}^{Nujol}$ 2.95, 3.18 μ (OH, NH), 6.28 (conj. C=O), Found: C, 64.75; H, 4.29; N, 6.67). The compound V was treated with diazomethane to yield an *O,O'*-dimethyl derivative (VI) (m.p. 187-188°; $\lambda_{max.}^{Nujol}$ 3.18 μ (NH), 6.18 (conj. C=O); found: C, 67.38; H, 5.45; N, 6.33; OCH_3, 26.55). III on hydrogenation over palladium-charcoal yielded trimethyldechloropyoluteorin (VII) (m.p. 137°; $\lambda_{max.}^{Nujol}$ 6.14 μ (conj. C=O), no OH-band, no NH-band; found: C, 68.68; H, 6.10; N, 5.98; OCH_3, 25.01; N-CH_3, 5.82).

Fusion of VII with potassium hydroxide resulted in the formation of 1,3-dimethoxybenzene (VIII) (b.p.$_{23}$ 105°; found: C, 69.82; H, 7.07) and N-methylpyrrole-2-carboxylic acid (IX) (m.p. 135°; found: C, 57.32; H, 5.42; N, 11.23).

The fact that alkali fusion furnished a product having a carboxyl group suggests that the carbonyl group is linked directly to both the benzene and the pyrrole rings. This was confirmed by reducing VII with lithium aluminum hydride, which led to 2,6-dimethoxyphenyl-N-methyl-2'-pyrrylmethanol (X) ($\lambda_{max.}^{EtOH}$ 241 mμ (ϵ 6,600) for the pyrrole ring, 271 mμ (ϵ 1,800) for the benzene ring; $\lambda_{max.}^{Nujol}$ 2.83 μ (OH), no conj. C=O band; not analyzed due to its instability). When VII was oxidized with chromium trioxide in acetic acid, the product was N-methyl-2,6-dimethoxybenzoylformamide (XI) (m.p. 143°; $\lambda_{max.}^{Nujol}$ 3.07 μ (NH), 5.87, 5.93, 5.99(conj. C=O), 6.56(NH); found: C, 59.34; H, 5.79; N, 6.33), which was also obtained by the similar treatment of III. XI was hydrolyzed with aqueous potassium hydroxide to the corresponding keto acid and the latter was further converted with hydrogen peroxide to 2,6-dimethoxybenzoic acid (XII) (m.p. 187°); which was identical with a synthetic sample. In view of the results obtained above, pyoluteorin must have the constitution I. The positions of the two chlorine atoms on the pyrrole ring will be clarified later.

$R_1 = R_2 = R_3 = H, X = Cl_2$ (I)
$R_1 = R_2 = COCH_3, R_3 = H, X = Cl_2$ (II)
$R_1 = R_2 = R_3 = CH_3, X = Cl_2$ (III)
$R_1 = R_2 = COCH_3, R_3 = H, X = H_2$ (IV)
$R_1 = R_2 = R_3 = H, X = H_2$, (V)
$R_1 = R_2 = CH_3, R_3 = H, X = H_2$ (VI)
$R_1 = R_2 = R_3 = CH_3, X = H_2$ (VII)



INSTITUTE FOR FERMENTATION ATTACHED TO THE TAKEDA PHARMACEUTICAL INDUSTRIES LTD. ROKURO TAKEDA
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RECEIVED JULY 14, 1958

THE RELEASE OF ZINC FROM CARBOXYPEPTIDASE AND ITS REPLACEMENT

Sir:

Carboxypeptidase is a zinc metalloenzyme and the metal is functional in enzymatic action.[1,2] 1,10-Phenanthroline (OP)[3] inhibits enzymatic activity and removes zinc from the protein[1]; spectral changes can be observed under such conditions.[4] We have now observed that zinc can be progressively removed by dialysis of carboxypeptidase at *p*H values between 5.5 and 3.4. As *p*H is lowered, increasing amounts of zinc are removed at an accelerating rate. Activity is abolished at a similar rate (CGP[3] as substrate). The correlation coefficient between metal content and activity is 0.90. Pertinent data are given in Table I.

TABLE I

THE LOSS OF ZINC AND PEPTIDASE ACTIVITY FROM CARBOXYPEPTIDASE IN ACID SOLUTION

Experimental conditions: 1.0*M* NaCl, 0.1*M* citrate, 2.8 mg./ml. protein, 0°; per cent. zinc and peptidase activity were measured after 48 hours dialysis at the given *p*H; $t_{1/2}$ gives the time required to reach 50% of the equilibrium values.

*p*H	Zn, % At equilibrium	Activity, % At equilibrium	$t_{1/2}$, hr. Zn	$t_{1/2}$, hr. Activity
5.46	..	86	..	25
5.22	68	71	20	24
4.98	52	76	11	11
4.48	30	46	12	13
3.92	17	19	8	8
3.38	5	8	3	2

Zinc also can be removed by dialysis against 2×10^{-3} *M* OP at *p*H 8.0,[5] 7.0 and 4.2. When OP is then removed by dialysis, and zinc is added back to the metal-free, inactive protein by dialysis at *p*H 7.0, its zinc content can be restored to 1 gram atom per mole with concomitant complete res-

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toration of activity. Addition of zinc in excess of 1 gram atom per mole does not result in additional activity and may inhibit; this excess zinc can be removed by dialysis at neutral *p*H. Similar results are obtained when the zinc is removed at *p*H 4.2, in the presence or absence of OP, and the protein further treated as above.

Dialysis of the metal-free protein against ions of Group IIA and IIB elements—Mg^{++}, Ca^{++}, Cd^{++}, and Hg^{++}—did not restore activity.

On the other hand, dialysis of the metal-free protein against ions of the first transitional period—Cr^{+++}, Mn^{++}, Fe^{++}, Co^{++}, and Ni^{++}—resulted in a significant *restoration* of activity.

The present experiments indicate the crucial nature of the metal atom as an active component of the enzymatic site of this exopeptidase. Carboxypeptidase can be isolated with its full complement of zinc, thus differing from many metal activated systems. This zinc can be successively removed and then be replaced. Therefore, carboxypeptidase constitutes a suitable model system to explore the significant difference in the physical-chemical and enzymatic properties of *metalloenzymes* and *metal-enzyme-complexes*.[6]

This work was supported by grants-in-aid from the National Institutes of Health of the Department of Health and Welfare.

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RECEIVED AUGUST 6, 1958

COENZYME Q: A NEW GROUP OF QUINONES[1]

Sir:

The isolation of crystalline quinone (Q-275) (I) from lipides of beef heart mitochondria has been described.[2,3,4] I acts as a coenzyme by undergoing cyclic oxidation and reduction during substrate oxidation in mitochondria.[2,5,6] Evidence bearing on the possible role of I in oxidative phosphorylation also has been presented.[7,8] *Anal.* Found: C, 82.24 ± 0.32 (8); H, 10.38 ± 0.27 (8); O, (Unterzaucher) 7.60, 7.71; O-Me, (Zeisel) 6.78 ± 0.16 (6); C-Me, (Kuhn-Roth) 13.4 ± 2.1 (3); moles H_2 absorbed per 100 g., 1.29; acetyl, zero; equiv. wt., 447; mol. wt., 900 and 910 (X-ray diffraction[9]), 779 (isothermal distillation), 527, 632, and 697 (ebullioscopic in butanone, acetic acid, ethanol, respectively); m.p. 49.9°; λ_{max}.275 mμ, 405 mμ, ethanol (oxidized form) and λ_{max}.290 mμ, ethanol (reduced form)[2]. By reductive acetylation the diacetate of dihydro I (m.p. 40°) (II) was prepared (*Anal.* Found: C, 79.41; H, 9.71; O-Me, 6.50, 7.08; acetyl, 9.96; moles H_2 absorbed per 100 g., 1.05; 1000 [X-ray], 1100 (isothermal distillation)). Levulinic acid, acetic acid and succinic acid have been identified as products of alkaline potassium permanganate treatment of I. A paraquinone function is indicated by the spectra of I and II. The other two oxygens are accounted for by two alkoxyl groups. A polyisoprenoid side chain(s) with one double bond per unit is indicated by the oxidation products and hydrogenation. X-Ray analysis[9] shows a pseudo-periodicity which is expected for such a side chain. The consumption of 11.1 moles of hydrogen, based on a molecular weight of 863,[10] indicates ten mono-unsaturated isoprenoid units.

This work has been confirmed and extended in accompanying communications.[10,11]

We also have succeeded in isolating from microbial sources four[12] other crystalline compounds which we believe to be homologs of I, differing from each other in the length of their polyisoprenoid side chains. On the basis of the formula $C_{59}H_{90}O_4$[10] for I, these compounds are formulated as III, $C_{54}H_{82}O_4$, Torula yeast, m.p. 45.2°; IV, $C_{49}H_{74}O_4$, *A. vinelandii*, m.p. 37°; V, $C_{44}H_{66}O_4$, Torula yeast, m.p. 30.5°; VI, $C_{39}H_{58}O_4$, *S. cerevisiae*, m.p. 16°. All data available support these conclusions. The ultraviolet and visible absorption spectra of all these compounds (I, III, IV, V, VI) are qualitatively identical, indicating a common quinonoid chromophoric group; equivalent weight (alkoxyl, oxygen, oxidation-reduction titration of hydroquinone) and molecular weight determinations (X-ray) confirm that these compounds differ from the next lower homologue by a five-carbon isoprenoid unit. In particular, cell dimensions[9] of I, III, IV substantiate this point. The estimate of the number of isoprenoid groups based on hydrogenation data would be 9.4, 8.0, 7.0 for III, IV and V, respectively.

Since all five quinones have coenzymatic activity,[5] we have altered our designation Q-275 to coenzyme Q to represent this new group of compounds. On the basis of the apparent number of isoprenoid units, we have designated the individual members of the coenzyme Q group as coenzyme Q_{10} (I); coenzyme Q_9 (III); coenzyme Q_8 (IV); coenzyme Q_7 (V), coenzyme Q_6 (VI).

Ubiquinone (SA) was described by Morton, *et al.*[13-16] as preparations (m.p. range 33-41°) from

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