preparation of CP[1] from frozen pancreas. It is demonstrated that purification of CP through recrystallization practically deletes the BGL and BGA splitting activity. Graded extraction of the euglobulin precipitate from *p*H 6.3 to *p*H 8.5 with barium hydroxide, shows decreasing ratios of BGL and BGA splitting activity relative to carbobenzyloxyglycyl-L-phenylalanine (CGP) splitting activity.

Action of BCP as a carboxypeptidase is demonstrated in its failure to hydrolyze benzoylglycyl-L-lysine amide.[2]

The existence of BCP as an enzyme distinct from CP is further demonstrated by the results obtained with crude euglobulin precipitate employing various competitive inhibitors (Table II). A CP inhibitor, 3-indolepropionate, does not inhibit the hydrolysis of BGL or BGA. ϵ-Aminocaproate and δ-aminovalerate, inhibit the hydrolysis of BGL and BGA and are without effect upon the hydrolysis of CGP. The inhibition of BCP by ϵ-aminocaproate and δ-aminovalerate suggests a similarity in mechanism of action of the two carboxypeptidases.[3]

TABLE II

EFFECT OF COMPETITIVE INHIBITORS ON THE HYDROLYSIS BY PANCREATIC EUGLOBULIN OF VARIOUS SUBSTRATES[a]

Inhibitor	Substrate CGP	BGL	BGA
None	30.0	25.5	24.5
3-Iodolepropionate,[b] 0.0025 *M*	11.5	25.7	25.0
ϵ-Aminocaprate,[c] 0.0025 *M*	30.0	0	7.5
δ-Amino-*n*-valerate[d] 0.0025 *M*	29.0	6.0	17.0

[a] Values reported as per cent. hydrolysis in 20 minutes. The enzyme concentration was 2.2×10^{-3} mg. of protein N/ml. Conditions for hydrolysis as reported in Table I. [b] Eastman Kodak Company, Rochester, New York, obtained as the free acid. [c] California Foundation for Biochemical Research, Los Angeles, California, obtained as the free acid. [d] California Foundation obtained as the acid mono HCl.

The *p*H optimum for partially purified BCP is 7.6 to 7.7. Its action is unaffected by natural occurring trypsin inhibitors and diisopropyl phosphorofluoridate. There is no observed hydrolysis of the following compounds after 24 hours with 0.3 mg. crude enzyme N/ml.: benzoyl-glycylnitro-L-arginine, α-benzoylglycyl-ϵ-carbobenzyloxy-L-lysine, carbobenzyloxyglycyl-L-proline, carbobenzyloxyglycyl-L-glutamic acid and carbobenzyloxy-β-glycyl-L-histidine. Details of purification, substrate specificity and inhibition of BCP as well as the synthesis of BGA will be reported subsequently.

The existence of a second type carboxypeptidase has been independently observed by Dr. J. Gladner and K. Laki. They find that upon incubation of DIP-trypsin (among other protein substrates) with CP (3X cryst. DFP-treated 50-fold molar excess, S:E = 25), one equivalent of lysine is rapidly released.[4]

(1) M. L. Anson, *J. Gen. Physiol.*, **20**, 663 (1937).
(2) K. Hofmann and M. Bergman, *J. Biol. Chem.*, **130**, 81 (1939).
(3) E. Elkins-Kaufman and H. Neurath, *ibid.*, **178**, 645 (1945).
(4) Personal communication, J. Gladner and K. Laki, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, manuscript in preparation.

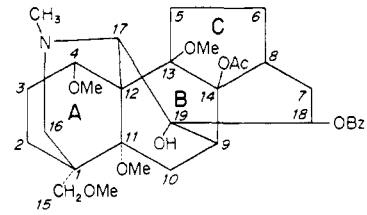
RESEARCH ASSOCIATE, AMERICAN DENTAL ASSOCIATION AT THE NATIONAL INSTITUTE OF DENTAL RESEARCH. NATIONAL INSTITUETS OF HEALTH, PUBLIC HEALTH SERVICE, U. S. DEPT. OF HEALTH, EDUCATION AND WELFARE BETHESDA, MD. J. E. FOLK

RECEIVED MAY 3, 1956

THE ACONITE ALKALOIDS. XXXII. THE STRUCTURE OF DELPHININE

Sir:

Recent developments, particularly infrared studies, lead us to propose for delphinine, $C_{33}H_{45}NO_9$, a hexacyclic modified diterpenoid structure (I)[1] which accommodates the following data. Oxidation furnishes α-oxodelphinine, containing an N-formyl group,[2,3] and β-oxodelphinine, most likely a δ-lactam (ν^{Nujol} 1645 cm.$^{-1}$). With the nitrogen contained in a six-membered ring,[4] the data suggest nitrogen bridging between the *gem*-methyl at C-16 and the angular methyl at C-17 as in some atisine alkaloids.[5-8] In β-oxadelphinine the CO would be at C-16. The formation of the



dicyclopentanobenzene hydrocarbon, $C_{17}H_{24}$, from hexahydrobenzoyloxodelphonine acetate[9] is explicable in terms of a contraction of ring A during dehydrogenation.[10]

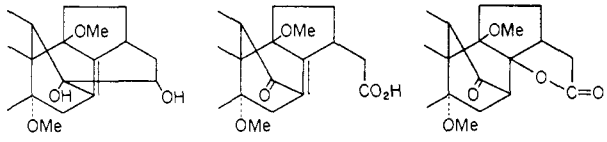
In order of lability[11] the first methoxyl group is tertiary and placed at C-11. Subsequent cleavage of the methoxyl at C-15 exposes a primary hydroxyl group which can be oxidized via an aldehyde to an acid, $C_{29}H_{33}NO_9$, whose methyl ester is resistant to hydrolysis.[12] The proximity of the methoxyls at C-11 and C-15 is shown by formation of oxido derivatives[11] accompanying demethylation of isopyro-α-oxodelphinine. The third methoxyl is tertiary and placed provisionally at C-13. Hydrolysis of the fourth methoxyl at C-4 exposes a secondary hydroxyl yielding desmethylanhydroisopyro-α-oxodelphinine. This can be oxidized *via* a six-membered ketone, $C_{27}H_{27}NO_7$ (ν^{KBr} 1712 cm.$^{-1}$), to the dicarboxylic acid, $C_{27}H_{27}NO_{10}$,[12] whose monomethyl ester (C-4) resists hydrolysis.

Hydrolysis of the acetoxy group placed at C-14

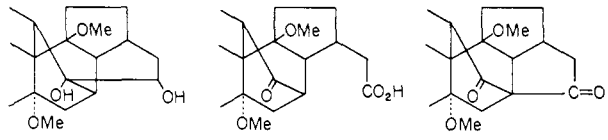
(1) To emphasize similarity to the diterpenes, our numbering system is based on that proposed for abietane by W. Klyne [*J. Chem. Soc.*, 3072 (1953)]. Our skeleton is formally derived from abietane by cleavage between C-6 and C-7 and closure between C-6 and C-8 with bridging of C-9 and C-17 by C-19.
(2) W. A. Jacobs and S. W. Pelletier, *Chem. and Ind.*, 948 (1955).
(3) The erroneous conclusion of W. Schneider [*Ann.*, **590**, 155 (1954)] that α-oxodelphinine is a δ-lactam was based on infrared absorption at 1668 cm.$^{-1}$. This absorption is due to its N-formyl group.[2]
(4) W. Schneider [*Arch. Pharm.*, **283**, 281 (1950)] obtained piperidine from delphinine with zinc dust.
(5) K. Wiesner, R. Armstrong, M. F. Bartlett and J. A. Edwards, *Chem. and Ind.*, 132 (1954).
(6) S. W. Pelletier and W. A. Jacobs, THIS JOURNAL, **76**, 4496 (1954).
(7) S. W. Pelletier and W. A. Jacobs, *Chem. and Ind.*, 1385 (1955).
(8) K. Wiesner and J. A. Edwards, *Experientia*, **11**, 255 (1955).
(9) W. A. Jacobs and C. F. Huebner, *J. Biol. Chem.*, **170**, 209 (1947).
(10) Precedent for such a contraction is available in the cevine series [W. A. Jacobs and S. W. Pelletier, *J. Org. Chem.*, **18**, 765 (1953); THIS JOURNAL, **78**, 1914 (1956)].
(11) W. A. Jacobs and Y. Sato, *J. Biol. Chem.*, **180**, 133 (1954).
(12) W. A. Jacobs and S. W. Pelletier, THIS JOURNAL, **76**, 161 (1954).

exposes a tertiary hydroxyl.[12] Pyrolytic loss of acetic acid furnishes unsaturated pyro compounds which in certain cases can be hydrogenated[9] and isomerized. Thus pyro-α-oxodelphinine ($\Delta^{8,14}$) isomerizes to isopyro-α-oxodelphinine ($\Delta^{9,14}$). Saponification of its benzoyl group gives isopyro-α-oxodelphonine (II), exposing a secondary hydroxyl vicinal to the free tertiary hydroxyl as shown by subsequent oxidation to an unsaturated keto acid $C_{24}H_{33}NO_8$ (III).[13]

Isomerization of the latter to the keto-γ-lactone



(IV) (ν^{CHCl_3} 1783, 1706 cm.$^{-1}$) can be explained by lactonization upon the $\Delta^{9,14}$ bond. Placing the benzoyloxy group at C-18 and the tertiary hydroxyl at C-19 satisfies these observations. Dihydroisopyro-α-oxodelphonine (V) on oxidation gives a ketoacid, $C_{24}H_{35}NO_8$ (VI) [ν^{Nujol}, (six-membered ketone), 1700 cm.$^{-1}$; methyl ester: ν^{Nujol} (CO_2Me), 1743 cm.$^{-1}$; (six-membered ketone), 1705 cm.$^{-1}$] which can be cyclized to a β-diketone,[14] $C_{24}H_{33}NO_7$ (VII) (ν^{KBr} 1766, 1720 cm.$^{-1}$). This may be reopened to the original acid. On catalytic reduction VII affords a hydroxy pentanone. (ν^{Nujol} 3409, 1746 cm.$^{-1}$).



For aconitine, which has two more hydroxyls than delphinine but bears an N-ethyl group, we envision the same basic skeleton since much of its chemistry parallels that of delphinine.

(13) W. A. Jacobs and Y. Sato, *J. Biol. Chem.*, **180**, 479 (1949).

(14) Neither the lycoctonine skeleton of Przybylska and Marion [*Canad. J. Chem.*, **34**, 185 (1956)] nor the related skeleton, derived from veatchine by the biogenetic pathway used by Valenta and Wiesner [*Chem. and Ind.*, 354 (1956)] to relate lycoctonine to atisine, accommodate all of our data.

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RECEIVED JUNE 11, 1956

FREQUENCY SHIFT OF CARBON-CHLORINE BOND VIBRATION IN METAL DERIVATIVES OF TETRA-(*p*-CHLOROPHENYL)-PORPHINE

Sir:

In a study of the infrared spectra of tetra-(*p*-chlorophenyl)-porphine and its metal derivatives,[1] we find (Table I) that the absorption band due to stretching of the C–Cl linkage will shift position depending upon whether the free porphine or a particular metal complex is under consideration.

Although Thomas and Martell[2] recently suggested that the C–Cl stretching frequency in these compounds might occur at 941 cm.$^{-1}$, the reference[3] upon which this suggestion was based indicates to us that this type of absorption would be expected to occur below 650 cm.$^{-1}$, and the higher value quoted by these authors is undoubtedly the first overtone of the stretching vibration.

TABLE I[a]

Porphyrin	ν_{C-Cl} (cm.$^{-1}$)
Free tetra-(*p*-chlorophenyl) porphine	491.0
Vanadyl salt	498.3
Copper salt	502.0
Cobalt salt	503.5
Nickel salt	504.5
Zinc salt	497.5

[a] Measured as KBr discs on a P. E. Model 12c Infrared Spectrophotometer equipped with KBr prism.

The structural formula of tetra-(*p*-chlorophenyl)-porphine (Fig. 1) indicates that the C–Cl linkages

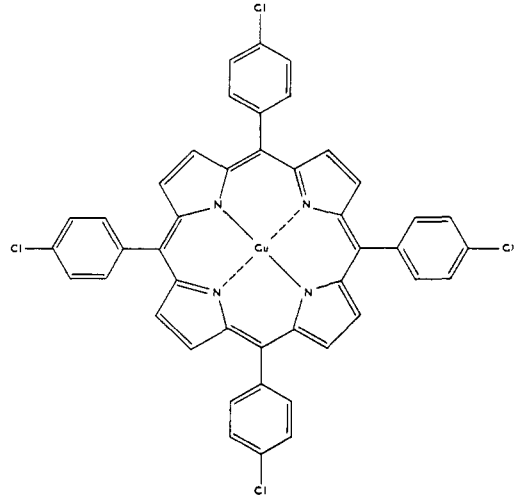

Fig. 1.

are relatively distant from the center of the porphine ring. Furthermore, the suggestion of these authors[2] that "the four benzene rings have partial rotation which cannot bring them 60° of being coplanar with the resonating porphine system" would indicate that the benzene rings themselves could not participate in resonance contributing forms of the porphine ring. Therefore, one might conclude that the C–Cl bond should not be affected by substituting a metal into the center of the molecule in place of the two hydrogen atoms of the free porphyrins. For these reasons, the observed frequency shifts of the C–Cl vibration seem somewhat contrary to expectation and may offer direct experimental evidence that the benzene rings are in fact in conjugation with the porphine nucleus. The higher vibrational frequencies of the metal derivatives seem indicative of more double bond character of the C–Cl linkage in these cases and, with this in mind, a great number of resonance structures can be postulated of which Fig. 2 may be taken as an example.

The hypothesis that the C–Cl bond character is altered by the introduction of a metal into the por-

(1) Prepared by method of P. Rothemund and A. R. Menotti, THIS JOURNAL, **63**, 268 (1941), and **70**, 1809 (1948). The vanadyl salt was prepared by method of J. T. Horeczy, *et al.*, *Anal. Chem.*, **27**, 1899 (1955).

(2) D. W. Thomas and A. E. Martell, THIS JOURNAL, **78**, 1338 (1956).

(3) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1952, p. 271.