$-62°$ ($CHCl_3$) [Calcd. for $C_{21}H_{30}O_4$ C, 72.80; H, 8.73. Found C, 72.74; H, 8.88 and C, 72.86; H, 8.74]. The assignment of configuration was based on rotatory power, taking as reference 4β,5-oxido coprostane-3-one, whose structure has been demonstrated.[1]

Rearrangement of the β- or α-epoxide with boron trifluoride in benzene yielded Δ^4-androstene-4,17β-diol-3-one, m.p. 222–223°, strong ferric chloride test, while acetic acid containing 2% of H_2SO_4 gave Δ^4-androstene-4,17-β-diol-3-one 17-acetate (4-hydroxytestosterone 17-acetate) m.p. 194–196°., $(\alpha)_D$ +83°, λ_{max} 277 mμ, ϵ = 12,100 [Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73; Found: C, 72.40; H, 8.80].

The latter compound formed with O-phenylenediamine a quinoxaline derivative and with acetic anhydride in pyridine a 4,17-diacetate, m.p. 170–172°, $(\alpha)_D$ +105°, λ_{max} 246 mμ, ϵ = 15,500 [Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.03; H, 8.25].

The 4β,5-oxidoetiocholane-17β-ol-3-one in acetone containing dil. H_2SO_4 rearranged to 2α-hydroxytestosterone, m.p. 160–162°, $(\alpha)_D$ +166° (reported[2]: m.p. 161–162°, $(\alpha)_D$ +120°), while the 4α,5-oxidoandrostane-17β-ol-3-one acetate gave the normal product of epoxide fission androstane-4β,5α,17β-triol-3-one 17-acetate, m.p. 213–215° [Calcd. for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85; Found: C, 69.07; H, 9.18].

Treatment of the 4β,5-oxidoetiocholane-17β-ol-3-one acetate with the requisite hydrogen halide in acetic acid formed the 4-bromotestosterone acetate, m.p. 196–197°, λ_{max} 261 mμ, ϵ = 11,600 [Calcd. for $C_{21}H_{29}O_3Br$: C, 61.61; H, 7.14. Found: C, 61.66; H, 7.18], the 4-chlorotestosterone acetate (3), m.p. 228–230°, $(\alpha)_D$ +118°, λ_{max} 255 mμ, ϵ = 13,300 [Calcd. for $C_{21}H_{29}O_3Cl$: C, 69.11; H, 8.01; Found: C, 69.37; H, 8.22] and the 4-fluorotestosterone acetate, m.p. 178–180°, λ_{max} 241 mμ, ϵ = 11,670 [Calcd. for $C_{21}H_{29}O_3F$; C, 72.38; H, 8.38. Found: C, 72.50; H, 8.61].

The reaction of the 4α,5-oxidoandrostane-17β-ol-3-one acetate with hydrogen chloride in acetic acid produced the 4β-chloroandrostane-5α,17β-diol-3-one-17-acetate, m.p. 202–204° [Calcd. for $C_{21}H_{31}O_4Cl$: C, 65.86; H, 8.15. Found: C, 65.68; H, 8.32] with on prolonged heating eliminated water to give 4-chlorotestosterone acetate.[3]

In the same way the α-epoxide acetate on reaction with hydrogen bromide in acetic acid yielded directly 4-bromotestosterone acetate.

The β- and α- epoxides reverted to testosterone on treatment with aqueous hydrogen iodide in chloroform or by heating with potassium iodide in acetic acid. The corresponding derivatives were obtained also from 11β-hydroxytestosterone, 17α-methyltestosterone, 19-nortestosterone, progesterone, 11β-hydroxyprogesterone, desoxycorticosterone, cortisone and other steroids and will be described in forthcoming papers.

(1) Pl. A. Plattner, H. Heusser and A. B. Kulkarni, *Helv. Chim. Acta*, **31**, 1822 (1948).

(2) F. Sondheimer, St. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, THIS JOURNAL, **75**, 4712 (1953).

(3) 4-Chlorotestosterone acetate was prepared also by direct chlorination of testosterone acetate.

Most of the described compounds showed remarkable anabolic activity and low androgenic effect. The following anabolic androgenic ratios, determined according to Hershberger, Shipley and Meyer,[4] at 500 γ daily doses, were obtained:

Testosterone propionate	0.28
4-Chlorotestosterone acetate	.88
4-Hydroxytestosterone acetate	.61
4-Fluorotestosterone acetate	.35
19-Nortestosterone cyclopentylpropionate	.72
4-Chloro-19-nortestosterone cyclopentylpropionate	1.82
4-Chloro-19-nortestosterone acetate	1.60

We are indebted to Dr. Sala and G. Baldratti of our Department of Pharmacology for the data on anabolic and androgenic potency and to Dr. F. Canal for the microanalyses.

(4) L. G. Hershberger, E. G. Shipley and R. K. Meyer, *Proc. Soc. Exp. Biol. Med.*, **83**, 175 (1953).

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A NEW PANCREATIC CARBOXYPEPTIDASE

Sir:

A hitherto unreported carboxypeptidase has been found in the euglobulin fraction obtained from bovine pancreas after autolysis. It manifests a rapid activity in releasing lysine and arginine from the carboxyl terminal position of the synthetic substrates, α-N-benzoylglycyl-L-lysine (BGL) and benzoylglycyl-L-arginine (BGA), which is not ascribable to the known carboxypeptidase (CP). The new enzyme is tentatively designated basic carboxypeptidase (BCP) because of its apparent requirement for a basic carboxyl terminal amino acid. Like CP it exists in fresh frozen pancreas as a proenzyme which is liberated in active form upon treatment of pancreas extract with trypsin.

In Table I are shown the proteolytic coefficients of some of the fractions obtained during a typical

TABLE I

PROTEOLYTIC COEFFICIENTS[a] OF CARBOXYPEPTIDASE PREPARATIVE FRACTIONS WITH DIFFERENT SUBSTRATES

	Substrate[b] CGP[c]	BGL[d]	BGA[e]
Crude pancreas extract	1.12	0.85	0.64
Crude euglobulin precipitate	3.26	2.55	1.90
Barium hydroxide extract	3.62	3.00	2.85
Once crystallized carboxypeptidase	18.00	2.85	2.10
Three times crystallized carboxypeptidase	19.40	[f]	[f]
Six times crystallized carboxypeptidase	19.10	[g]	[g]

[a] First order proteolytic coefficient defined as the first order velocity constant, $Kt = \log (a/a - x)$, per mg. protein N/ml. The extent of hydrolysis was measured on 0.2-ml. samples by the alcoholic KOH titration procedure of Grassman and Heyde, *Z. physiol. Chem.*, **183**, 32 (1929). [b] Substrates employed at 0.025 *M* concentration with 0.025 *M* "tris" buffer *p*H 7.65 at 25°. [c] Hofmann and Bergmann, *J. Biol. Chem.*, **134**, 225 (1940). [d] Hofmann and Bergmann, *ibid.*, **134**, 225 (1940); Folk, *Arch. Biochem. Biophys.*, in press. [e] Folk, unpublished data. [f] Five to 10% hydrolysis in 24 hours at a substrate–enzyme molar ratio of 300 to 1. [g] Very little hydrolysis, only detectable by paper chromatography after 24 hours at a substrate–enzyme molar ratio of 30 to 1.

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.

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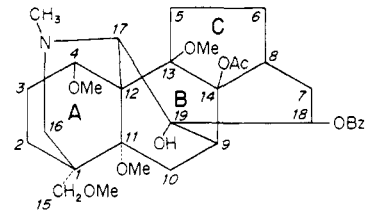
THE ACONITE ALKALOIDS. XXXII. THE STRUCTURE OF DELPHININE

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).