of release of free amino acids indicated that the N-terminal sequence was Asp(-NH$_2$)-Ser-Cys-Glu-Gly and this was confirmed by the isolation of a series of labeled peptides from the aminopeptidase digest lacking progressively Asp(-NH$_2$), Asp(-NH$_2$) Ser, Asp(-NH$_2$)Ser CySO$_3$H and Asp(-NH$_2$)Ser CySO$_3$H Glu, as indicated in Fig. 1 (top). In accordance with the specificity of trypsin, the C-terminal residue of the peptide was found to be lysine, using carboxypeptidase B.[4]

PARTIAL ACID HYDROLYSIS OF S-8 PEPTIDE BY 12 N HCl AT 37°C FOR 72 HRS.

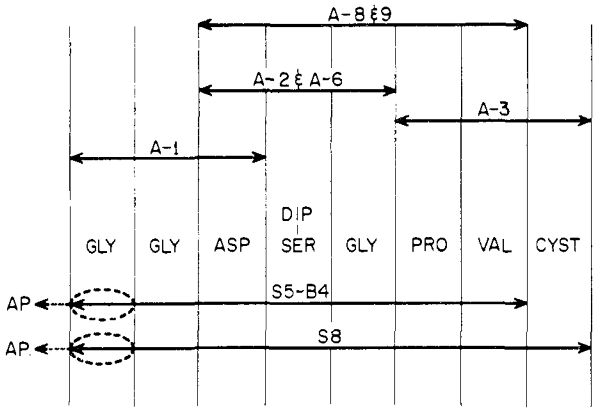

Fig. 1.—Enzymatic degradation of the peptide O-Tr-1: the horizontal arrows delineate the peptides obtained by degradation with subtilisin (S) or aminopeptidase (AP). The vertical arrows denote the major points of cleavage by subtilisin. The sequence denoted by "Army" has been established previously by the authors of ref. 5. Peptides S6 and S7 were present in trace quantitities only and their radioactivities were below the limits of detection.

The remainder of the sequence was determined by subtilisin digestion which yielded 14 peptides. Of the subtilisin peptides, only S5-B4, S8 and S9-B4 were radioactive, and their composition showed in each case the presence of a single serine. Glycine was liberated from both S5-B4 and S8 by aminopeptidase, while neither peptide gave any free amino acid with carboxypeptidase, probably due to the C-terminal cysteic acid (S8) and the penultimate proline (S5-B4). S8 was subjected to partial acid hydrolysis and the resulting peptides could be fitted into a unique sequence (Fig. 2). In Fig. 1, these subtilisin peptides are combined with the sequences found by aminopeptidase and acidic hydrolysis, and provide a unique sequence. The sequence Asp-Ser-Gly around the DIP-seryl confirms that previously determined by Schaffer, *et al.*[5]

Much interest has been focused recently upon the similarity in composition around the phosphorylated serine in those enzymes inhibited by DFP.[6]

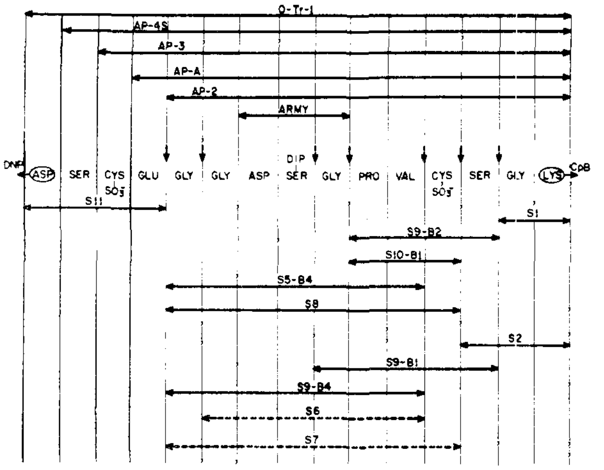

Fig. 2.

Detailed sequences are, however, available only for chymotrypsin[7] and trypsin (above) and these show complete identity over the sequence Gly-Asp-$\overset{\text{DIP}}{\text{Ser}}$-Gly. Recently, Westheimer[8] has attempted to explain in detail the orientation of the active site of chymotrypsin by assuming the folding of this hypothetical sequence

$$\text{—X—X—X—His—X—Gly—Asp—}\overset{\text{DIP}}{\text{Ser}}\text{—Gly—Glu—}\atop\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\text{Ala—Val—}$$

in an α-helix, thus bringing the histidine and serine into the favorable orientation previously suggested by Cunningham.[1,9] On the basis of our evidence for the sequence in trypsin which possesses an essentially identical bond-breaking mechanism, the assumption as to the position of the histidine is unjustified. In addition, the presence of two cysteic residues (and proline) close to the serine would probably preclude the formation of an α-helix in this region. The absence of histidine from the trypsin peptide described above (and in fact even from the largest peptide containing 55 residues) would suggest that maintenance of the histidine and serine in a favorable orientation is a question of the tertiary structure of the protein.[10]

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[4] J. E. Folk, THIS JOURNAL, **78**, 3541 (1956).

[5] N. K. Schaffer, R. E. Engle, L. Simet, R. W. Drisko and S. Harshman, *Fed. Proc.*, **15**, 347 (1956).

[6] N. K. Schaffer, S. C. May and W. H. Summerson, *J. Biol. Chem.*, **202**, 69 (1953); R. A. Oosterbaan, H. S. Jansz and J. A. Cohen, *Biochim. et Biophys. Acta*, **20**, 402 (1956); G. H. Dixon, S. Go and H. Neurath, *ibid.*, **19**, 193 (1956); D. E. Koshland, Jr., and M. J. Erwin, THIS JOURNAL, **79**, 2657 (1957); F. Turba and G. Gundlach, *Biochem. Z.*, **327**, 186 (1955).

[7] N. K. Schaffer, L. Simet, S. Harshman, R. R. Engle and R. W. Drisko, *J. Biol. Chem.*, **225**, 197 (1957).

[8] F. H. Westheimer, *Proc. Nat. Acad. Sci.*, **43**, 969 (1957).

[9] L. W. Cunningham, *Science*, **125**, 1145 (1957).

[10] G. H. Dixon and H. Neurath, *Biochim. et Biophys. Acta*, **20**, 572 (1956).

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RECEIVED JANUARY 15, 1958

ON THE ABSOLUTE CONFIGURATION OF THE ANTIBIOTIC ACTIDIONE

Sir:

The striking results of optical rotary dispersion measurements among polycyclic ketones[1] have

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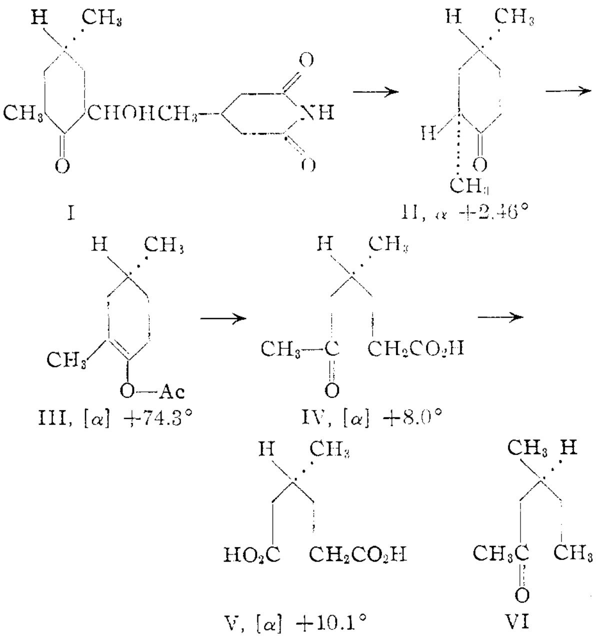
[1] For leading references see C. Djerassi, *Bull. Soc. Chim. France*, 741 (1957), and C. Djerassi, O. Halpern, V. Halpern, O. Schindler and C. Tamm, *Helv. Chim. Acta*, **41**, No. 1 (1958), in press.

prompted us to extend such studies to optically active cyclohexanones. For the proper evaluation of the dispersion data it was important to use cyclohexanones of known absolute configurations and these are generally best secured from terpenes. Thus, (+)-3-methylcyclohexanone is readily obtained[2,3a] from (+)-pulegone and serves as an extremely useful standard for many stereochemical correlations[3] and for transformations to substituted cyclohexanones for conformational studies by rotatory dispersion measurements.[4]

For these reasons it would be very desirable to have accessible additional alkylated cyclohexanones with known absolute configurations and the present communication deals with (+)-2,4-dimethylcyclohexanone (II). This ketone is formed in one step by alkaline treatment of the antibiotic actidione (I),[5] which is prepared commercially in large amounts because of its agricultural applications. The determination of the absolute configuration of II affords a reference standard for the eventual elucidation of the absolute configuration of the remaining asymmetric centers of this antibiotic[6] and even more importantly provides a convenient model and starting material for experimental and theoretical rotatory dispersion studies as will become apparent from subsequent papers.

(+)-2,4-Dimethylcyclohexanone (II)[5] ($\alpha^{25}\text{D}$ +2.46°, neat) exhibits a single negative Cotton effect curve[7] in methanol solution (*c*, 0.097) with a trough at 297.5 mμ (−278°) and a peak at 275 mμ (−57°) and was transformed into its enol acetate III[8] (b.p. 48° (1.5 mm.), $[\alpha]^{25}\text{D}$ +74.3° (octane); *Anal.* Found for $C_{10}H_{16}O_2$: C, 71.03; H, 9.95). Ozonolysis provided (+)-4-methyl-6-oxoheptanoic acid (IV) (b.p. 101° (0.02 mm.), $[\alpha]^{25}\text{D}$ +8.0° (CHCl_3), $\lambda_{\max}^{\text{CHCl}_3}$ 5.80 μ; *Anal.* found for $C_8H_{14}O_3$: C, 60.10; H, 8.05; neut. equiv., 165) whose single positive Cotton effect curve[7] was opposite in sign to that of (+)-2-ethyl-4-pentanone (VI). Since the latter had been synthesized[9] from (−)-2-ethyl-1-propanol of known[10] absolute configuration (S),[11] the 4-methyl group of II presumably[12] belongs to the D-series (*R* according to the new convention[11]).

Rigorous confirmation was provided by hypobromite oxidation of IV leading to (+)-β-methyladipic acid (V) which already had been related[3a,13] to D-glyceraldehyde. Since (+)-2,4-dimethylcyclohexanone (II) was formed under alkaline conditions the two methyl groups of II can be assumed to be *cis* from which it follows that the absolute configuration (2R:4R[11]) as depicted in II correctly represents (+)-*cis*-2,4-dimethylcyclohexanone.



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(2) O. Wallach, *Ann.*, **289**, 337 (1896).

(3) (a) See E. J. Eisenbraun and S. M. McElvain, THIS JOURNAL, **77**, 3382 (1955); (b) A. Melera, D. Arigoni, A. Eschenmoser, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, **39**, 441 (1956).

(4) C. Djerassi, L. E. Geller, J. Osiecki and E. J. Eisenbraun, paper to be presented at "Conformational Analysis" Symposium, ACS, San Francisco meeting, April, 1958.

(5) E. C. Kornfeld, R. G. Jones and T. V. Parke, THIS JOURNAL, **71**, 150 (1949). We are indebted to Dr. E. C. Kornfeld (Eli Lilly and Company) and Dr. D. I. Weisblat (Upjohn Company) for supplies of actidione.

(6) This may also be of help in synthetic studies—see D. D. Phillips, M. A. Acitelli and J. Meinwald, *ibid.*, **79**, 3517 (1957).

(7) For nomenclature see C. Djerassi and W. Klyne, *Proc. Chem. Soc.*, 55 (1957).

(8) The presence of some of the double bond isomer is not excluded.

(9) L. E. Geller, unpublished observation in these laboratories.

(10) L. Crombie and S. H. Harper, *J. Chem. Soc.*, 2685 (1950).

(11) R. S. Cahn, C. K. Ingold and V. Prelog, *Experientia*, XII, 81 (1956).

(12) This is predicated on the assumption that the carboxyl group of IV can be ignored which turned out to be justified.

(13) K. Freudenberg and W. Hohmann, *et al.*, *Ann.*, **584**, 54 (1954).

(14) (a) Postdoctorate research fellow on funds supplied by the National Science Foundation; (b) Predoctorate research fellow on funds supplied by the National Cancer Institute (Grant No. CY-2919) of the U. S. Public Health Service.

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RECEIVED FEBRUARY 3, 1958

SOME ISOMORPHOUS TERNARY OXIDES CONTAINING TANTALUM

Sir:

In attempts to make $\text{Ba}_{0.5}\text{TaO}_{2.5}$ and similar compounds, we have prepared some ternary oxides containing tantalum which have a somewhat different composition. From powder and single crystal X-ray data, these compounds appear to be isomorphous.

Mixtures were made according to equations (1) and (2) and heated at 1250° in evacuated, sealed capsules for three 24-hour periods. The samples were reground between heatings.

$$0.50\text{BaO} + 0.40\text{Ta}_2\text{O}_5 + 0.20\text{Ta} = \text{Ba}_{0.50}\text{Ta}^{\text{IV}}\text{O}_{2.5} \quad (1)$$

$$0.50\text{BaO} + 0.20\text{Ta}_2\text{O}_5 + 0.10\text{Ta} + 0.50\text{NbO}_2 = \text{Ba}_{0.50}(\text{Ta}_{0.50}{}^{\text{IV}}\text{Nb}_{0.50}{}^{\text{IV}})\text{O}_{2.5} \quad (2)$$

Two analyses of the product of reaction (1) for barium and tantalum, plus a determination of weight gain on heating in air, indicated the composition $\text{Ba}_{0.44}(\text{Ta}_{0.74}{}^{\text{IV}}\text{Ta}_{0.26}{}^{\text{V}})\text{O}_{2.57}$. The product of reaction (2) has not been analyzed.

When reaction (1) was run with the reactants wrapped in tantalum foil, platy blue crystals