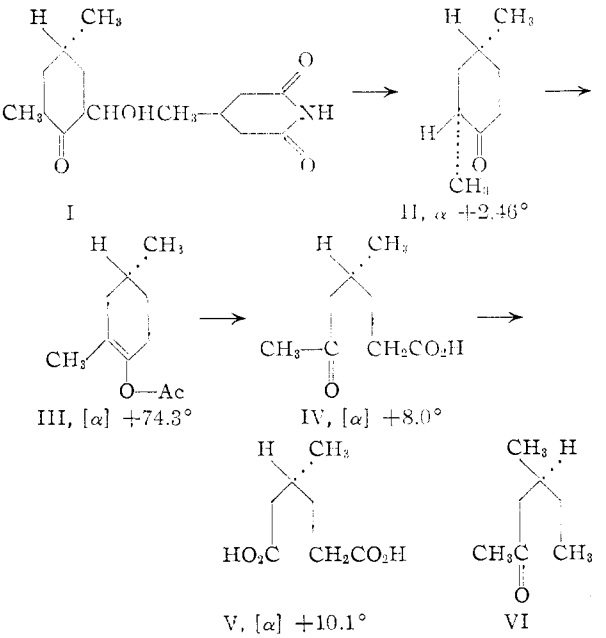
prompted us to extend such studies to optically active cyclohexanones. For the proper evaluation of the dispersion data it was important to use cyclohexanones of known absolute configurations and these are generally best secured from terpenes. Thus, (+)-3-methylcyclohexanone is readily obtained[2,3a] 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] ($\alpha^{25}D$ +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.), $[\alpha]^{25}D$ +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.), $[\alpha]^{25}D$ +8.0° ($CHCl_3$), $\lambda_{max}^{CHCl_3}$ 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.



(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 $Ba_{0.5}TaO_{2.5}$ 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.

$$0.50BaO + 0.40Ta_2O_5 + 0.20Ta = Ba_{0.50}Ta^{IV}O_{2.5} \quad (1)$$

$$0.50BaO + 0.20Ta_2O_5 + 0.10Ta + 0.50NbO_2 = Ba_{0.50}(Ta_{0.50}^{IV}Nb_{0.50}^{IV})O_{2.5} \quad (2)$$

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 $Ba_{0.44}(Ta_{0.74}^{IV}Ta_{0.26}^{V})O_{2.57}$. The product of reaction (2) has not been analyzed.

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

measuring as much as 0.8 mm. in greatest dimension were formed. Precession photographs of the zero and first levels taken with the X-ray beam perpendicular to the plate, showed the crystals to be hexagonal (Laue symmetry D_{6h}-6/mmm). Further examination revealed no systematic absences, so the probable space group is one of P622, P6mm, ($P\bar{6}m2$, $P\bar{6}2m$), or P6/mmm. Cell dimensions as found from the powder photograph are $a = 8.96$ Å. and $c = 7.79$ Å.

The preparation containing niobium is dark blue. The X-ray powder pattern is indexable on the basis of a hexagonal cell with $a = 9.01$ Å. and $c = 7.81$ Å.

An attempt to substitute Ge^{IV} for Nb^{IV} in reaction (2) was not successful. It was discovered, however, that mixing reactants according to equation (3) resulted in a product structurally similar to those of reactions (1) and (2).

$$0.75BaO + 0.25Ta_2O_5 + 0.50GeO_2 = Ba_{0.75}(Ge_{0.50}^{IV}Ta_{0.50}^{V})O_{3.0} \quad (3)$$

When a small sample of the preparation containing germanium was cooled slowly, clear hexagon-shaped crystals were formed. Precession photography showed these crystals to have the same Laue symmetry and space group possibilities as the crystals of reaction (1). Cell dimensions as found from the precession and powder photographs are $a = 8.96$ Å. and $c = 7.79$ Å. The product has not been analyzed chemically.

Work is being carried out to determine the structure of these compounds using the crystals prepared by reaction (1). The electrical properties are also being examined. From preliminary measurements on a pressed pellet of the niobium compound, it was found to be an *n*-type conductor and to have thermoelectric properties. A plot of resistivity versus temperature from 70 to 145° produced a straight line with the resistivity increasing with temperature.

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RECEIVED JANUARY 14, 1958

THE ACTIVE SITE OF THROMBIN

Sir:

In previous communications[1,2] we have reported the inactivation of thrombin by diisopropylphosphorofluoridate (DFP) and on the stoichiometry of the reaction. We now wish to report the isolation of a series of radioactive peptides from acid hydrolyzed P^{32}-labeled diisopropylphosphoryl (DIP) thrombin. These experiments identify serine as the site of the DFP binding and a sequence of Asp.-Ser.Gly as the amino acids about the serine residue.

(1) J. A. Gladner and K. Laki, *Arch. Biochem. and Biophys.*, **62**, 501 (1956).

(2) J. A. Gladner, K. Laki and F. Stohlman, *Biochim. et Biophys. Acta*, in press.

Thrombin was inactivated by DFP^{32} and purified by column chromatography as previously described.[2] The enzyme was degraded by mild acid hydrolysis, and P^{32}-containing peptides were isolated by column chromatography on Dowex-50 as described by Schaffer, *et al.*[3] Qualitative and quantitative amino acid analysis of the peptides was performed utilizing the two-dimensional paper chromatographic system of Irreverre and Martin.[4] Compositions of these phosphopeptides are shown in Table I.

TABLE I

RADIOACTIVE PHOSPHOPEPTIDES ISOLATED FROM DIP^{32}-THROMBIN

Fraction of peptide	Amino acid composition
2	(Asp, Ser, Gly)
4	(Asp, Ser)
5	(Ser, Gly)
7	(Asp, Ser, Gly)
8	(Asp, Ser, Gly, Glu, Ala)

All amino acids present in the individual fractions 2 through 7 were in a molar ratio of 1; glycine in fraction 7, however, was present in a greater proportion. The yield of fraction 8 was too small to estimate proportions accurately. Quantitative column chromatography according to the method of Moore and Stein,[5] of fraction 2, performed by Dr. D. R. Kominz (this laboratory), gave a molar ratio of Asp:Ser:Gly = 1:0.96:0.93. End group analysis (N-terminal) of fraction 2, carried out by Dr. J. E. Folk, National Institute of Dental Research, yielded aspartic acid. Since serine is common to all fractions, it is evident that this amino acid residue contains the bound phosphate. The data establish the sequence Asp.Ser.-Gly for fraction 2.

The fact that the radioactive fractions which emerged from the Dowex-50 column corresponded identically in number, volume displacement, distribution and amino acid content to those observed by Schaffer, *et al.*,[3] for DIP^{32}-chymotrypsin, make it very likely that the position of the second glycine in fraction 7 is N-terminal, thus giving the sequence Gly.Asp.Ser.Gly. It is also noteworthy that the composition of each peptide is in excellent agreement with the over-all presumed sequence Gly.Asp.Ser.Gly.Glu.Ala. reported for chymotrypsin[3,6] and the Asp.Ser.Gly sequence found in trypsin.[6,7] The possibility that this agreement occurs by chance is exceedingly remote.

The amino acid compositions of the fractions are also in remarkable agreement with these reported by Koshland and Erwin for the active site of phosphoglucomutase.[8] We wish to postulate, therefore, the sequence Gly.Asp.Ser.Gly(Glu, Ala) as a portion of the active site of thrombin.

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

(4) F. Irreverre and W. Martin, *Anal. Chem.*, **26**, 257 (1954).

(5) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 633 (1951).

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

(7) N. K. Schaffer, *J. Biol. Chem.*, personal communication, in press.

(8) D. E. Koshland and M. J. Erwin, THIS JOURNAL, **79**, 2657 (1957).