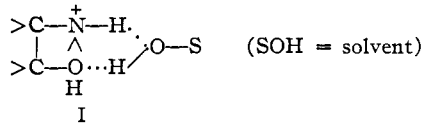
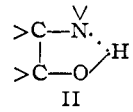
to be. In view of Fodor and Nador's result, it was our opinion that the base strengths of the tropanols do not represent what would be expected, but are actually anomalous in comparison with the behavior of analogously constituted systems; that is, the *a priori* interpretation of the base strengths would, on logical grounds, lead to the conclusion opposite to what is now known to be the correct one; their interpretation in terms of the known structures rests upon *post hoc* arguments.

Prelog and Häfliger[7] showed that there exist distinct differences in the pK values of pairs of 1,2-aminoalcohols, the *cis*-oid isomer being the stronger base. Prelog and Häfliger attributed this difference to the fact that hydrogen bonding stabilized the *cis*-oid conjugate acid (I) and thus made the *cis*-oid base appear the stronger (its conjugate acid the weaker) of such a *threo–erythro* pair. While it



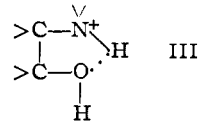
I

may be argued *a priori*, as Smith and Hartung appear to have done, that hydrogen bonding could stabilize the base with respect to protonation (II), making the *cis*-oid member the weaker base, and thus direct the argument to the opposite conclusion,



II

Prelog and Häfliger's results in the case of the ephedrine–pseudoephedrine series, interpreted in the light of structure assignments arrived at in other ways,[8] lead to the conclusion that the possibility of hydrogen bonding between the amino and hydroxyl groups increases base strength. Pauling[9] and Trotman-Dickenson[10] have discussed hydrogen-bonded solvation of amines and ammonium ions and have concluded that hydrogen bonding is more effective in the ions than in the free bases.[11] From these considerations it is reasonable to conclude that if a structure such as II is regarded as possible, the sterically equivalent III would be equally likely, and thus the stabilization of the ion III should contribute to increasing the strength of the base, as Prelog and Häfliger conclude.



III

In order to examine this question further, pK values were determined for four amines, *dl-cis*- and *trans*-2-aminocyclohexanol and *dl-cis*- and *trans*-2-aminocyclopentanol.[12] The results (Table II) are entirely in accord with the conclusions of Prelog and Häfliger. The *cis*- and *trans*-aminocyclohexanols and the *cis*-aminocyclopentanol are of about equal strength as bases and are stronger (by about 0.4 pK unit) than *trans*-aminocyclopentanol.

TABLE II

dl-1,2-Aminocycloalkanol	pK_A
cis-6	9.72
cis-5	9.70
trans-6	9.63
trans-5	9.28

In view of the foregoing evidence it would appear to be the logical conclusion that tropine, the stronger base, has the *cis* configuration and that pseudotropine is *trans*. Fodor and Nador[2] have shown, however, that the reverse is true.

It can be concluded that a sound interpretation of the differences between the base strengths of tropine and pseudotropine cannot be based upon a consideration only of the configuration of the hydroxyl group. Still unresolved effects of the conformations of these substances in solution may be of importance, possibly with respect to inter- rather than intramolecular interactions. It is possible that the strain imposed upon the ring system by the ethylene bridge is affected by the position of the hydroxyl group with respect to the bridge, and thus the tendency for the nitrogen atom to assume the tetrahedral form by ionization is affected by the configuration of the hydroxyl group. These questions cannot be answered without further study of suitably constituted models. This work is in prospect.

Experimental

Samples of tropine and pseudotropine were prepared by known methods. The compounds were rigorously purified by distillation and recrystallization. The pure substances had m.p. 63–64° (tropine) and 110–110.5° (pseudotropine).

Piperidine was redistilled; a middle, constant-boiling (107.2–107.3° (760.1 mm.)) fraction was used.

Titrations were carried out under nitrogen. Precautions were taken to exclude access of air to the purified bases. The standard acid and base used in the titrations were carefully standardized.

Calculation of the half-neutralization point from the weight of sample used, or by selecting the mid-point of the completed titration curve always gave values that agreed to within less than the differences between successive runs, and usually within 0.01 pK unit. In Table I no attempt has been made to define the statistical limits of error, but it is probable that the average values given are correct to ± 0.05 pK unit.

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(7) V. Prelog and O. Häfliger, *Helv. Chim. Acta*, **33**, 2021 (1950).

(8) W. J. Close, *J. Org. Chem.*, **15**, 1131 (1950).

(9) L. Pauling, "Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1949.

(10) A. F. Trotman-Dickenson, *J. Chem. Soc.*, 1293 (1949).

(11) See also, G. E. K. Branch and M. Calvin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1941, p. 229.

(12) We are indebted to Dr. W. E. McCasland for his generosity in furnishing us with samples of these four compounds.

Stereochemical Specificity of Enzymatic Cleavage of β-Phenylserine

BY JAMES B. GILBERT

RECEIVED APRIL 28, 1954

The enzymatic cleavage of several β-hydroxy-α-amino acids,[1] including β-phenyl-DL-serine,[2] of unspecified configuration, to glycine and an aldehyde has been described. However, the rate of splitting

(1) A. E. Braunshtein and G. Ia. Vilenkina, *Doklady Akad. Nauk S.S.S.R.*, **66**, 243 (1949).

(2) G. Ia. Vilenkina, *ibid.*, **69**, 385 (1949).

of the four separate isomers of a β-hydroxy-α-amino acid by this enzyme has not been reported. This is herein accomplished for β-phenylserine.[3] The cleavage of β-phenylserine is of additional interest due to the structural and possible metabolic relationships of the compound with epinephrine and chloroamphenicol.

When a purified rat liver preparation was employed as enzyme source, it was found (Table I) that *erythro*-β-phenyl-L-serine was rapidly cleaved at nine times the rate of *threo*-β-phenyl-L-serine. The respective D isomers were not measurably attacked under the conditions employed. This result regarding the *threo* isomers of β-phenylserine is analogous to the data obtained using racemic and L-threonine which indicated that L-threonine was selectively attacked.[4]

TABLE I

ENZYMATIC CLEAVAGE OF ISOMERS OF β-PHENYLSERINE

Enzymatic digests consisted of 2 cc. of 0.1 *M* borate buffer to which was added 1 cc. of substrate and 1 cc. of purified rat liver enzyme.[a] Incubated at 37° for 15 min. Enzymatic splitting of substrate was linear with respect to time over intervals reported.

Additions		Mg. N/cc.		μmoles formed/hr./mg. N	
μmoles	Substrate	enzyme	*p*H	Benzaldehyde[b]	Glycine[c]
5	*threo*-β-Phenyl-L-serine	0.27	8.3	2.4	
5	*threo*-β-Phenyl-D-serine	.62	8.3	<0.1	
5	*erythro*-β-Phenyl-L-serine	.04	8.4	21.6	
5	*erythro*-β-Phenyl-D-serine	.62	8.4	<0.1	
20	DL-Allothreonine[d]	1.03	8.1		3.1
20	*erythro*-β-Phenyl-DL-serine[d]	0.23[e]	8.0	27.0	28.6

[a] The enzyme was purified as described under Experimental. [b] Benzaldehyde determined directly on the enzymatic digest by measuring the increment in optical density at 250 mμ using a Model DU Beckman spectrophotometer.[5] [c] Glycine determined by ninhydrin decarboxylation and colorimetric determination of formaldehyde formed with chromatropic acid.[6] [d] Incubated 30 min. [e] Enzyme preparation more active.

erythro-β-Phenyl-L-serine was split at seven times the rate obtained with DL-allothreonine as substrate (Table I). The relative rate of cleavage was similar in a crude rat liver extract. Therefore, *erythro*-β-phenyl-L-serine is more rapidly cleaved by this enzyme than previously examined substrates.[1] This result also suggests that the β-phenyl-DL-serine employed in earlier enzymatic studies[2] was of the *threo* configuration. The rapid splitting of the *erythro* isomer coupled with the ease of determination of this cleavage[5] should facilitate the further purification of the enzyme. The *p*H optima for enzymatic cleavage of *erythro*-β-phenyl-DL-serine and DL-allothreonine were *p*H 8.3 and *p*H 8.0, respectively.

Benzaldehyde was identified as a product of the cleavage of *erythro*-β-phenyl-DL-serine, by steam distillation of the enzymatic digest and isolation as the 2,4-dinitrophenylhydrazone, m.p. 239.5° (cor.), mixed m.p. 239.5° (cor.). *Anal.* Calcd. for $C_{13}H_{10}N_4O_4$: N, 19.58. Found: N, 19.73. It was also demonstrated that glycine was formed in equimolar quantity with benzaldehyde (Table I).

As a preliminary step in ascertaining the effect of substitution of β-hydroxy-α-amino acids, α-methyl-DL-serine[3,7] was incubated with pigeon liver extract possessing activity with respect to other β-hydroxy-α-amino acids. No evidence of alanine formation was detected on paper chromatograms of the enzymatic digest.

Experimental

Enzyme Fractionation.—Rat livers were homogenized in a blendor with two volumes of cold water. The resulting extract was centrifuged at 42,000 times gravity for two hours. An equal volume of a saturated aqueous solution of ammonium sulfate was added to the supernatant. The resulting precipitate was taken up in a minimum volume of water and dialyzed against water at 5° until free of ammonia. The dialyzed solution was centrifuged and the precipitate discarded. The supernatant solution was diluted to a concentration of 1.5 mg. N/cc. To this solution were added, in order, an equal volume of 0.02 *M* sodium acetate solution and a half volume of an aged calcium phosphate gel[8] (dry weight: 35 mg./cc. of initial suspension). After standing for one hour the suspension was centrifuged and the enzyme eluted from the gel with 0.01 *M* phosphate buffer *p*H 7.3. This enzyme solution was dialyzed against running water at 5° for two hours and lyophilized. The activity of the purified rat liver enzyme was 20 μ moles of benzaldehyde formed/hr./mg. N when *erythro*-β-phenyl-DL-serine was employed as substrate. This preparation represented a five times purification of the original extract and 8% recovery of the original total activity.

Acknowledgment.—The author wishes to thank Dr. Jesse P. Greenstein for his advice and encouragement.

(3) Kindly donated by Dr. William S. Fones of this Laboratory; W. S. Fones, *J. Biol. Chem.*, **204**, 323 (1953). α-Methyl-DL-serine was synthesized by method of J. Billman and E. E. Parker, THIS JOURNAL, **67**, 1069 (1945).

(4) G. Ia. Vilenkina, *Doklady Akad. Nauk S.S.S.R.*, **84**, 559 (1952).

(5) C. W. Tabor, H. Tabor and S. M. Rosenthal, *J. Biol. Chem.*, **208**, 645 (1954).

(6) H. N. Christensen, T. R. Riggs and N. E. Ray, *Anal. Chem.*, **23**, 1521 (1951).

(7) *dextro*-α-Methylserine has been identified recently as a constituent of the antibiotic Amicetin; E. H. Flynn, J. W. Hinman, E. L. Caron and D. O. Woolf, THIS JOURNAL, **75**, 5867 (1953).

(8) N. K. Sarkar and J. B. Sumner, *Enzymologia*, **14**, 280 (1951).

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4-Nitro- and 4-Amino-3-picoline

BY WERNER HERZ AND LIN TSAI

RECEIVED APRIL 9, 1954

In the course of other work in this Laboratory it was desirable to study the preparation of certain 4-substituted 3-picolines. It is well known that electrophilic substitution on the pyridine nucleus invariably introduces a group into the β-position, whereas similar reactions on pyridine 1-oxide have been shown by Ochiai and his school[1] and independently by den Hertog and co-workers[2] to proceed in a different manner. Thus nitration of pyridine 1-oxide leads primarily to 4-nitropyridine 1-oxide which may subsequently be reduced to 4-nitropyridine. By a similar method we have

(1) For a review of work by the Japanese investigators, see E. Ochiai, *J. Org. Chem.*, **18**, 534 (1953).

(2) H. J. den Hertog and W. P. Combe, *Rec. trav. chim.*, **70**, 581 (1951); H. J. den Hertog and J. Overhoff, *ibid.*, **69**, 468 (1950); H. J. den Hertog, C. R. Kolder and W. P. Combe, *ibid.*, **70**, 591 (1951); H. J. den Hertog and W. P. Combe, *ibid.*, **71**, 745 (1952); H. J. den Hertog, C. H. Henkens and J. H. van Room, *ibid.*, **71**, 1145 (1952); H. J. den Hertog, C. H. Henkens and K. Dilz, *ibid.*, **72**, 298 (1953).