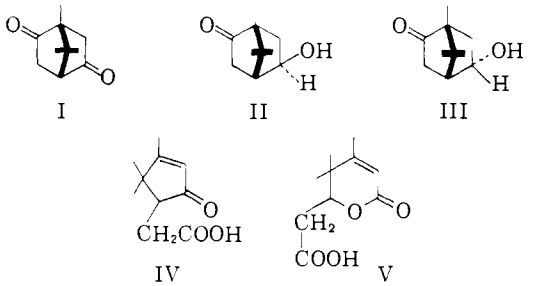
COMMUNICATIONS TO THE EDITOR

MICROBIOLOGICAL DEGRADATION OF (+)-CAMPHOR

Sir:

Clarification of the biosynthesis of complex organic structures, *e.g.*, terpenoids and alkaloids, is proceeding rapidly. In contrast the processes of biodegradation which are intrinsic to the continual redistribution of carbon in Nature generally have been ignored. This paper describes some initial studies on the microbiological decomposition of (+)-camphor.

A pseudomonad, strain P, isolated from sewage sludge by enrichment techniques using (+)-camphor as a carbon source, was grown on a medium containing (+)-camphor (0.5%) and minerals.[1] Extraction of the broths at the end of the logarithmic growth phase and chromatography of the neutral fraction yielded (1) a keto-camphor identified as 2,5-diketocamphane (I) by comparison with an authentic sample,[2] (2) a hydroxyketone, m.p. 220.5–221.5°, $[a]^{EtOH}_D$ + 41° (*c*, 71.48; H, 10.60), identified as 5-*exo*-hydroxycamphor (II)[3] by the physical properties, the correspondence of *p*-nitrobenzoate,[4] 3,5-dinitrobenzoate[5] and semicarbazone[4] derivatives, and oxidation to I,[6] and (3) other hydroxyketones (oxidizable to I) including 5-*endo*-hydroxycamphor (III) isolated as the *p*-nitrobenzoate, m.p. 147–148° (C, 64.45; H, 6.05; N, 4.26), the structure of which was clarified by n.m.r.



The acid fraction afforded a keto acid, $C_{10}H_{14}O_3$, m.p. 104–108°, $[a]^{EtOH}_D$ −57°, $\nu_{max}^{CHCl_3}$ 1705, 1620 cm.$^{-1}$, $\lambda_{max}^{C_2H_5OH}$ 227 mμ(ε, 14,000), (*c*, 65.80; H, 7.92; neut equiv., 183), equilibrated by heating in aqueous solution to a racemate m.p. 125–127°, dihydro derivative, m.p. 83–84°, $\nu_{max}^{CHCl_3}$ 1735, 1710 cm.$^{-1}$ (C, 65.15; H, 9.20). These spectral features together with n.m.r. data on the methyl ester [peak at 0.475[7] from C=CHCO, four methyl peaks at +1.65, +3.30, +4.15 and +4.38 and a 3-proton multiplet at +2.7] suggested structure IV and identity was established by comparison with authentic IV.[8]

Complete oxidation of the acid IV by the resting bacterial cells is inhibited by 2,2′-bipyridine with the accumulation of a new intermediate, $C_{10}H_{14}O_4$, m.p. 105.5–107°, $[a]^{CHCl_3}_D$ +57° (*c*, 60.62; H, 7.16; 32.01; neut. equiv., 201), $\lambda_{max}^{H_2O}$ 220 mμ (ε, 9750). n.m.r. (peaks at −0.45, +0.80 (triplet), +2.58 (doublet), +3.30, +4.15, +4.22) and I.R. ($\nu_{max}^{CHCl_3}$ 1710, 1726 cm.$^{-1}$) spectra indicated structure V. This assignment was confirmed by hydrogenation to a dihydro lactone, m.p. 135–136°, $[a]^{CHCl_3}_D$ −17.5°, infrared and nuclear magnetic resonance spectra identical with the lactone from racemic dihydro IV and peracetic acid, m.p. 129–129.5° (C, 59.97; H, 7.84).

The pathway by which camphor is degraded by this organism can be partially formulated as: (+)-camphor → 5-hydroxycamphor → I → IV → V, a succinct process for the cleavage of both carbocyclic rings.[9]

(1) R. Y. Stanier, M. Doudoroff and E. A. Adelberg, "General Microbiology," The Macmillan Co., New York, N. Y., 1958, p. 286.

(2) J. Bredt and A. Goeb, *J. prakt. Chem.*, **101**, 288 (1921).

(3) See Y. Asahina and M. Ishidate, *Ber.*, **64**, 188 (1931).

(4) M. Ishidate, H. Kawahata and K. Nakazawa, *ibid.*, **74**, 1707 (1941).

(5) F. Reinhartz and W. Zanke, *ibid.*, **67**, 552 (1934).

(6) The configuration at C_5 is assigned from the n.m.r. absorption of the 5-proton which occurs as a triplet in the *p*-nitrobenzoate (5.5 cps. band spacing). See *e.g.*, M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959). See also K. Takeuchi, *Sci. Pap. Inst. Phys. Chem. Res. Tokyo*, **23**, 288 (1933).

(7) Chemical shift in p.p.m. relative to external CH_2Cl_2 in solvent $CDCl_3$.

(8) J. Bredt and P. Pinten, *J. prakt. Chem.*, **119**, 81 (1928); Y. Asahina and M. Ishidate, *Ber.*, **67**, 440 (1934).

(9) Supported in part by the National Science Foundation.

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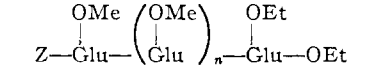
CONFORMATIONAL TRANSITIONS IN POLYPEPTIDES

Sir:

We wish to report the discovery of a conformational transition in low molecular weight polypeptide chains. Optically active peptides of the type



(where *n* is an integer between zero and seven and *Z* is the benzyloxycarbonyl group) have been prepared.[1] The rotations of the compounds were taken in dichloroacetic acid and in dioxane (Fig. 1). In dichloroacetic acid values were negative and decreased, approaching those found for the high molecular weight polymers in this solvent.[2] In dioxane a similar plot was obtained for the di-, tri- and tetrapeptides. However, at the pentamer stage the rotation suddenly shifted to a positive value and continued to rise as the residues increased. For high molecular weight glutamic acid esters positive rotations have been attributed to helical forms.[3]

(1) Details of the synthesis will be published elsewhere. The general method is described in M. Goodman and K. C. Stueben, THIS JOURNAL, **81**, 3980 (1959).

(2) E. R. Blout, R. H. Karlson, P. Doty and B. Hargitay, *ibid.*, **76**, 4492 (1954).

(3) (a) C. Robinson and M. S. Bott, *Nature*, **168**, 325 (1951); (b) P. Doty and J. T. Yang, THIS JOURNAL, **78**, 498 (1956); (c) J. T. Yang and P. Doty, *ibid.*, **79**, 761 (1957); (d) P. Doty and R. D. Lundberg,

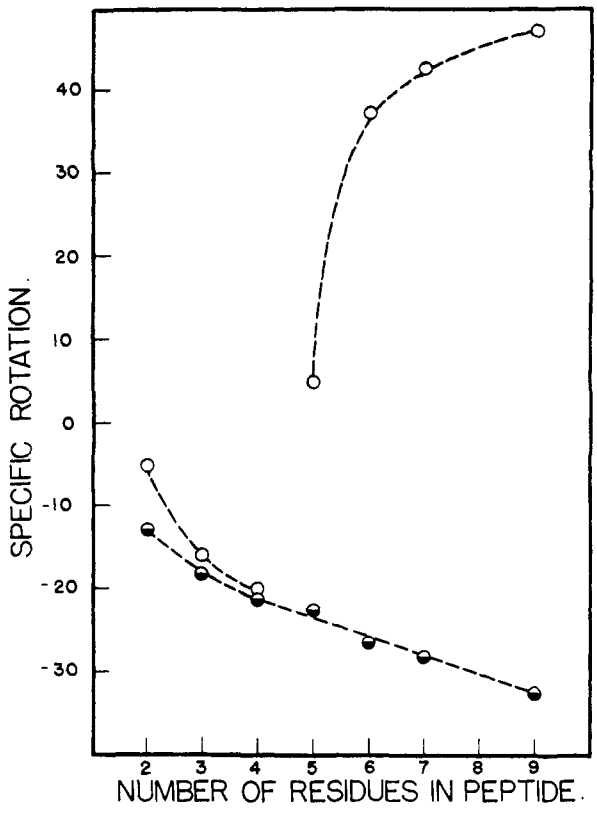


Fig. 1.—Optical activity of peptide derivatives as a function of solvent and number of residues. All rotations were measured in dioxane (open circles) and dichloroacetic acid (half-filled circles) at 2% concentration except the hepta- and nonapeptides in dioxane solution. These rotations were measured on a 1.43% and 0.22% solution, respectively.

Another explanation for positive optical rotations has been offered by Blout and Doty[4] for low molecular weight peptides. By polymerizing the γ-benzyl-α-amino acid anhydride of glutamic acid they obtained polydisperse oligomeric γ-benzyl-L-glutamate ($\overline{DP}$ 5.2), and found a concentration and solvent dependent positive rotation. This they ascribe to an associated β-form in solution.

The optical activities in dioxane of our di-, tri- and tetrapeptides were completely independent of concentration even at concentrations as high as 20%. On the other hand, the pentapeptide and hexapeptide showed decided concentration dependencies [+5° at 3% to −29° at 0.1%], and [+35 at 2% to 0° at 0.05%], respectively, while the heptamer and nonamer exhibited little or no concentration dependence.

In addition, a second solid form of these pure oligomers has been isolated by the technique of carefully heating the hexamer or higher in dioxane until precipitation occurred. The resulting solid is completely insoluble in dioxane. By heating, most probably, hydrogen bonds are broken and the peptide chain rearranges in a manner analogous to protein denaturation.[5] The chain is then able to associate as a β-extended structure which is insoluble in dioxane. This second solid form can be reconverted to the original by dissolving it in a hydrogen bond breaking solvent such as N,N-dimethylformamide and precipitating the peptide with ethanol.

We suggest the alternate possibility that the positive rotations for these oligomeric peptides are due to formation of intramolecular hydrogen bonds of the type found in the α-helix[6] of high molecular weight polyglutamic acid esters. The di-, tri-, and tetrapeptides indicate no detectable association or enhanced optical activity above that found for the solvated random coil in dichloroacetic acid. The penta and higher peptides, on the other hand, show both association[7] and enhanced optical rotations. On this basis we believe that the association is dependent upon a prior folding of the peptide chain.

Acknowledgment.—We gratefully acknowledge the support for this research given by the National Association of Glue Manufacturers.

(4) P. Doty, A. M. Holtzer, J. H. Bradbury and E. R. Blout, *ibid.*, **76**, 4493 (1954).

(5) C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides," Academic Press, Inc., New York, N. Y., 1956, p. 333.

(6) L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci.*, **37**, 241 (1951).

(7) Poly-γ-benzyl glutamates also exhibit association in solvents such as chloroform and dioxane; P. Doty, J. H. Bradbury and A. M. Holtzer, THIS JOURNAL, **78**, 947 (1956).

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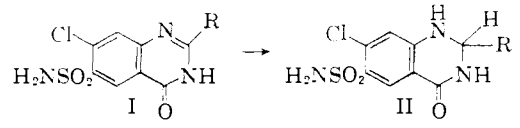
QUINAZOLINONE SULFONAMIDES AS DIURETIC AGENTS

Sir:

In view of the great current interest in orally active diuretic agents, which are neither organic mercurial compounds nor primarily carbonic anhydrase inhibitors,[1,2] we wish to report on a series of highly active 7-chloro-6-sulfamyl-4(3H)-quinazolinones (I) and 7-chloro-6-sulfamyl-1,2,3,4-tetrahydro-4-quinazolinones (II).



These compounds cause a marked natriuresis and chloruresis in rats and dogs on oral administration, but at the same time cause only a relatively small increase in potassium excretion.

In general, minor variations in R from H to lower alkyl have very little effect on the over-all activity observed in this series but do affect slightly the dose-response curves and the Na^+, Cl^- and K^+ excretion ratios as observed experimentally.[3]

Conversion of the quinazolinones (I) to the 1,2,3,4-tetrahydroquinazolinones (II) results in an enhancement of oral diuretic activity on a dose/kg.

(1) F. C. Novello and J. M. Sprague, THIS JOURNAL, **79**, 2028 (1957).

(2) Annals of the New York Academy of Sciences, "Chlorothiazide and Other Diuretic Agents," Vol. 71, pp. 321–478 (1958).

(3) We are indebted to Dr. J. R. Cummings and his associates of the Experimental Therapeutics Research Section, Pearl River Laboratories, for the pharmacological data reported.