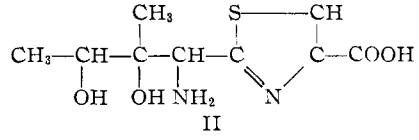
COMMUNICATIONS TO THE EDITOR

DEGRADATION OF THIOSTREPTON.[1] THE STRUCTURE OF THIOSTREPTINE

Sir:

Paper chromatography of acid hydrolyzates of the antibiotic thiostrepton[2] reveals several ninhydrin positive components, namely cyst(e)ine, thiostreptoic acid,[3] threonine, 2-aminomethylthiazole-4-carboxylic acid[4] (I), alanine, isoleucine and trace amounts of glycine. If the hydrolysis in 5.7 *N* hydrochloric acid at 110° is interrupted after 5 hours, only a small amount of I can be detected on paper chromatograms. Instead, a new component, *thiostreptine*, appears, to which structure II is assigned on the basis of these considerations.



II

Thiostreptine moves somewhat faster than alanine in a 1-butanol–acetic acid–water (4:1:5) system, and like I gives a yellow color with ninhydrin, which slowly changes to purple.[5] If the hydrolysis time is extended, the intensity of the spot corresponding to II decreases with a simultaneous increase in the intensity of the spot for I, until after about 40 hours II is no longer present.[6]

In order to isolate II, thiostrepton was hydrolyzed with a 1:1 mixture of formic acid and concentrated hydrochloric acid[7] at 110° for 5 hours. After evaporation to dryness the residue was dissolved in water, extracted with ether and the aqueous layer subjected to countercurrent distribution in a system of 1-butanol–ethanol–0.1% acetic acid (4:1:5). After 1200 transfers II was detected in a band corresponding to $K = 0.2$. Removal of the solvents gave crude II, which then was purified further by partition chromatography on a cellulose powder column in a system of 1-butanol–acetic acid–water (4:1:1). An amorphous but chromatographically homogeneous product was obtained; $[\alpha]_D$ −4° (*c*, 1.0 in *N* AcOH). *Anal.* Calcd. for $C_9H_{14}O_4N_2S$: C, 43.9; H, 5.7; N, 11.4; S, 13.0; neutr. equiv., 246; C-methyl, 12.6. Found: C, 45.6; H, 6.7; N, 11.4; S, 13.7; neutr. equiv., 233 (as base), 230 (as acid); C-methyl (Kuhn–Roth) 15.1; λ_{max}^{alc} 237 mμ (ϵ = 6800) essentially identical with that of I.[4]

The analytical values indicate that II was not completely pure. A crystalline salt from II with 4-hydroxyazobenzene-4′-sulfonic acid[8] turned out to be unstable. When stored at room temperature it was converted slowly into the salt of I. Therefore it was decided to continue the degradation studies with the above mentioned amorphous material.

On oxidation with periodate II consumed 2 moles of the reagent. From the reaction mixture approximately 1 mole of acetaldehyde was isolated in the form of its dinitrophenylhydrazone and identified by comparison with an authentic sample (m.p., mixed m.p., infrared spectra, paper chromatography[8] and analysis). About 1 mole of acetic acid was formed in the oxidation, isolated as the sodium salt and identified by paper chromatography[9] and infrared spectrum. The third product isolated by extraction of the acidified mixture with ether and sublimation was shown to be 2-formylthiazole-4-carboxylic acid (III): III has no well-defined m.p. and is reducing toward tetrazolium reagent. *Anal.* Calcd. for $C_5H_3O_3NS$: C, 38.2; H, 1.9; N, 8.9; S, 20.3; mol. wt., 157. Found: C, 38.9; H, 2.2; N, 8.0; S, 20.0; mol. wt. (Rast), 170; λ_{max}^{alc} 234 mμ; λ_{max}^{KBr} 5.92 μ shoulder at 5.85 μ. The n.m.r. spectrum of III in CH_3CN shows one aldehydic proton (τ 0.83) and an aromatic proton (τ 2.85). The mass spectrum[10] demonstrates the presence of one sulfur and one nitrogen atom and of a COOH group and indicates a molecular weight of 157.

When thiostreptine was treated with constant boiling HCl for 30 hours the hydrochloride of I was isolated in good yield and identified by comparison (m.p., paper chromatography, infrared spectra) with a synthetic sample obtained from Professor Kenner's Laboratory.[4] Furthermore, acetaldehyde and acetoin were isolated from this reaction as the dinitrophenylhydrazone and osazone,[11] respectively.

Confirmation of structure II for thiostreptine was obtained by n.m.r. spectroscopy. In trifluoroace-

(1) For previous paper in this series, see *J. Am. Chem. Soc.*, **83**, 3906 (1961).

(2) (a) J. Vandeputte and J. Dutcher, "Antibiotics Annual 1955–1956," Medical Encyclopedia, Inc., New York, N. Y., p. 560; (b) J. F. Pagano, M. J. Weinstein, H. A. Stout and R. Donovick, *ibid.*, 1955–1956, p. 554; (c) B. A. Steinberg, W. P. Jambor and Lyda O. Suydam, *ibid.*, 1955–1956, p. 562.

(3) M. Bodanszky, J. T. Sheehan, J. Fried, N. J. Williams and C. A. Birkhimer, *J. Am. Chem. Soc.*, **82**, 4747 (1960).

(4) G. W. Kenner, R. C. Sheppard and C. E. Stehr, *Tetrahedron Letters*, 23 (1960).

(5) Thiostreptine was first thought (ref. 3, footnote 9) to be identical with 2-(1′-aminopropyl)-thiazole-4-carboxylic acid, isolated from thiostrepton by Kenner and co-workers (ref. 4). This later was found to be in error.

(6) Quantitative assay of 16 hour hydrolyzates by a modified Stein–Moore procedure, based on a molecular weight of 1600, shows 1 mole of threonine, 2 moles of alanine, 1 mole of isoleucine, 4.4 moles of ammonia, only minor amounts of II, of thiostreptoic acid, of glycine, about 0.45 mole of I and about 0.3 mole of cystine.

(7) G. L. Miller and V. du Vigneaud, *J. Biol. Chem.*, **118**, 101 (1937).

(8) A. I. Schepartz, *J. of Chromatography*, **6**, 185 (1961).

(9) In a system of 1-butanol–ethanol–3 *N* NH_4OH (4:1:5), *cf.* F. Brown, *Biochem. J.*, **49**, 598–600 (1950).

(10) The authors wish to express their gratitude to Professor Klaus Biemann of M.I.T. for the mass spectrum and its interpretation.

(11) Acetoin, when treated with dinitrophenylhydrazine in 2 *N* HCl, gives the osazone, m.p. *ca.* 235° dec. (*Anal.* Calcd. for $C_{16}H_{14}O_8N_8$: N, 25.1. Found: N, 24.7), identical (infrared spectrum, chromatography on a thin layer of Al_2O_3) with the bisdinitrophenylhydrazone of biacetyl. Acetaldehyde and acetoin also are formed when thiostrepton is treated with 0.5 *N* NaOH at room temperature and the clear solution obtained is distilled. Acetaldehyde was removed from the distillate with a stream of air which in turn was led through a solution of dinitrophenylhydrazine in 2 *N* HCl. The resulting precipitate was identified as acetaldehyde dinitrophenylhydrazone. In the distillate from which acetaldehyde was removed acetoin was identified by oxidation with $FeCl_3$ to biacetyl and conversion of the latter into nickel dimethylglyoxime (A. J. Kluyver, H. J. Donker and F. Vissert Hooft, *Biochem. Z.*, **161**, 361 (1925)).

tic acid,[12] thiostreptine shows a singlet (3 protons, 2′-methyl) at τ = 8.7, a doublet (3 protons, ω-methyl) centered at τ = 8.4, J = 6 and single protons at τ = 6.4 (3′-H), 4.4 (1′-H) and 1.2 (5-H).

Thiostreptine can be regarded as derived from β,γ-dihydroxyisoleucine and cysteine. It is interesting to note its relationship to the antibiotic hydroxyaspergillic acid,[13] which is a derivative of β-hydroxyisoleucine.

(12) The n.m.r. spectrum was taken soon after preparing the solution. After a few days at room temperature it had changed considerably, and on paper chromatograms a second as yet unidentified spot, yellow with ninhydrin and turning purple, was observed. The same component was detected when solutions, or even solid samples of II, were stored at room temperature.

(13) J. D. Dutcher, *J. Biol. Chem.*, **232**, 785 (1958).

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NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY. LONG-RANGE SPIN COUPLINGS IN VINYLACETYLENE[1]

Sir:

The failure of long-range spin coupling to be observed in the high-resolution proton magnetic resonance spectra of vinylacetylene and propargylaldehyde has been cited[2] as evidence against

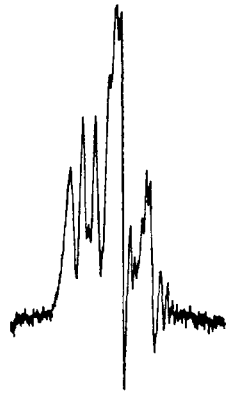

Fig. 1.—Splitting of acetylenic proton resonance of vinylacetylene in benzene solution at room temperature at 60 Mc. taken with Varian A-60 spectrometer. The splitting between the extreme peaks is 2.05 ± 0.05 cps. The appearance of the spectrum is very sensitive to the chemical shifts of the vinyl protons.

hyperconjugation as a mechanism for long-range coupling in unsaturated compounds. The reasoning involved has been challenged recently by Hoffman and Gronowitz[3] on theoretical grounds.

It is our belief that further theoretical discussions of this subject must take into account the fact that the couplings in question are by no means negligibly small (*i.e.*, <0.5 cps.) as reported.[2] This is clearly illustrated by Fig. 1 which shows the n.m.r. spectrum of the acetylenic proton of vinylacetylene in benzene solution under very high resolution (<0.20 cps.). The couplings which correspond to the observed splittings are J_{13} = −2.17 cps., $J_{14}(cis)$ = 0.70 cps., and $J_{14}(trans)$ = 0.92 cps. (all ± 0.05 cps.) for vinylacetylene[4] and 0.58 ± 0.05 cps. for propargylaldehyde. The reality of the long-range couplings in vinylacetylene has been further confirmed beyond any question by the proton spectrum of $CH_2{=}CHC{\equiv}CD$, which substance shows the calculated simplification of the vinyl resonances expected for reduction of the magnitude of the relevant couplings by a factor of seven (the H to D gyromagnetic ratio).

A complete analysis of the n.m.r. spectrum of vinylacetylene and its monodeuterated analog will be published later.

(1) Supported in part by the Office of Naval Research and the Undergraduate Research Participation Program of the National Science Foundation.

(2) M. M. Kreevoy, H. B. Charman and D. R. Vinard, *J. Am. Chem. Soc.*, **83**, 1978 (1961).

(3) R. A. Hoffman and S. Gronowitz, *J. Am. Chem. Soc.*, **83**, 3910 (1961).

(4) The signs of the J values are relative to positive signs being taken for couplings within the vinyl group.

(5) National Institutes of Health Postdoctoral Fellow, 1959–61.

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SQUARE PLANAR-TETRAHEDRAL ISOMERISM AMONG SOME COMPLEXES OF NICKEL(II). A NEW TYPE OF GEOMETRIC ISOMERISM

Sir:

Considerable attention has been given in recent years to the structure of nickel(II) complexes and, as a result, a number of tetrahedral[1] or distorted tetrahedral[2,3] complexes have been characterized, while most of the earlier supposed examples of such complexes have been shown to contain nickel(II) in various octahedral environments. For example, $[NiX_2(P\text{-}n\text{-}Bu\text{-}Ph_2)_2]$ (X = Cl, Br, I) recently were found to be paramagnetic (μ_{eff} = 3.2–3.4 B.M.) and tetrahedral (probably distorted) in the crystalline state. It was thought that the structure of the complexes in solution was either distorted tetrahedral or *cis*-planar and that the magnetic and spectral properties of the solutions could be accounted for in terms of the thermal population of two neighboring energy states, one corresponding to a diamagnetic and the other to a paramagnetic molecule. An alternative interpretation in terms of an equilibrium between a *trans*-planar diamagnetic form and a tetrahedral (or *cis*-planar) paramagnetic form was thought to be less likely.[2]

We have investigated recently the reactions of Ph_2EtP with nickel halides and, from ethanolic solution, have obtained the complexes (I)–(III), (Table I).

An interesting feature of the series (I)–(III) is that the chloride alone is diamagnetic and thus square planar in the solid state. On the other hand, the bromide and iodide are paramagnetic and hence have tetrahedral structures both by analogy with the corresponding complexes of Ph_3P[2] and

(1) N. S. Gill and R. S. Nyholm, *J. Chem. Soc.*, 3997 (1959).

(2) (a) M. C. Browning, R. F. B. Davies, D. J. Morgan, L. E. Sutton and L. M. Venanzi, *ibid.*, 4816 (1961), and previous work by Venanzi, *et al.*, *ibid.*, 2705 (1961); 719 (1958).

(3) D. M. L. Goodgame, M. Goodgame and F. A. Cotton, *J. Am. Chem. Soc.*, **83**, 4161 (1961), and references therein.