

1-Piperidyl-2- α -naphthylcarbonyl-3,4,6-triacetyl-D-glucose

A 7.5-g. portion of 1-piperidyl-3,4,6-triacetyl-D-glucose¹ was dissolved in 75 ml. of anhydrous pyridine. A 4.2-g. portion of α -naphthyl isocyanate was added, the flask was stoppered, and the mixture allowed to stand for an hour. At the end of this period, the flask was placed on a steam-bath for 15 minutes and then maintained at room temperature overnight. The solution was then diluted with 10 ml. of water and heated on the steam-bath for 10 minutes, whereupon it was poured into 300 ml. of ice-water. The sirup formed at this point became crystalline on maceration with 25 ml. of cold methanol. Crystallization of the crude product from 300 ml. of methanol yielded 10 g. (90%) of

(1) J. F. Hodge and C. E. Rist, *THIS JOURNAL*, **74**, 1498 (1952).

essentially pure product melting at 162–163°. Two additional crystallizations yielded a pure product melting at 164°, $[\alpha]_D^{20} +36.0^\circ$ (c 1.5, chloroform).

Anal. Calcd. for $C_{28}H_{34}O_9N_2$: C, 61.98; H, 6.34; N, 5.16. Found: C, 62.11; H, 6.49; N, 5.02.

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COMMUNICATIONS TO THE EDITOR

CHARACTERIZATION OF THE ANTIBIOTIC
THIOLUTIN AND ITS RELATIONSHIP WITH
AUREOTHTRICIN

Sir:

In 1950, the antibiotic Thiolutin was described as a neutral, optically inactive, yellow-orange crystalline elaboration product of *Streptomyces albus* to which the tentative empirical formula $C_{13}H_{14}N_3O_3S_3$ was assigned.¹ Subsequently, it became apparent that the description of aureothricin, $C_{13}H_{13}N_3O_3S_3$, previously isolated in Japan, bore much resemblance to Thiolutin.² Accordingly, in 1950 an interchange of samples was arranged³ and preliminary comparisons confirmed the similarities of the two antibiotics. However, recent chemical studies on Thiolutin led to the preparation of significant degradation products and their derivatives which further characterize this antibiotic and differentiate it from aureothricin. The present work invalidates the original empirical formulas of Thiolutin and aureothricin and characterizes their molecular formulas as $C_8H_8N_2O_2S_2$ and $C_9H_{10}N_2O_2S_2$, respectively.

Raney nickel desulfurization of Thiolutin in ethanol yields a white crystalline product designated "desthiolutin" (I) m.p. 130–131°. *Anal.* Calcd. for $C_8H_{14}N_2O_2$: C, 56.45; H, 8.29; N, 16.46; mol. wt., 170. Found: C, 56.78; H, 8.24; N, 16.19; mol. wt. (Rast) 187, (Signer) 160.

Cautious acid hydrolysis of Thiolutin liberates one mole of acetic acid to yield a monoamine (II), amorphous free base, m.p. 191–194° (dec.), crystalline hydrochloride hydrate salt,⁴ sinters $\sim 200^\circ$, $\lambda_{\text{inf}}^{\text{mu}}$ 229, ϵ 5400; $\lambda_{\text{max}}^{\text{mu}}$ 309, ϵ 6100; $\lambda_{\text{max}}^{\text{nu}}$ 381, ϵ 11,000. *Anal.* Calcd. for $C_6H_8N_2OS_2 \cdot HCl \cdot H_2O$:

(1) F. W. Tanner, Jr., J. A. Means and J. W. Davisson, Abstracts 118th Meeting, American Chemical Society, September 7–8, 1950.

(2) (a) H. Umezawa, K. Maeda and H. Kosaka, *Japanese Medical Journal*, **1**, 512 (1948); (b) H. Umezawa, T. Tazaki, K. Maeda, H. Kosaka and S. Fukuyama, *Journal of Antibiotics (Japan)*, **2**, Suppl. A, 105 (1949); (c) K. Maeda, *ibid.*, **2**, 795 (1949); (d) K. Maeda, *Japanese Medical Journal*, **2**, 85 (1949).

(3) Aureothricin (m.p. 260–270° dec., see note 5) was obtained through the courtesy of Dr. H. Umezawa.

(4) Light absorption determined on methanol solutions.

C, 29.94; H, 3.77; N, 11.64; S, 26.62; Cl, 14.72; H_2O , 7.49; neut. equiv., 240.7. Found: C, 30.22; H, 3.86; N, 11.67; S, 26.86; Cl, 15.06; H_2O , 7.62; neut. equiv., 239.5 (water-ethanol).

The amino compound (II) has been further characterized by a variety of crystalline acylated derivatives: acid succinamido compound (III) m.p. 254–255° (dec.); *Anal.* Calcd. for $C_{10}H_{10}N_2O_4S_2$: neut. equiv., 286. Found: neut. equiv., 282, 290 (dimethylformamide-water); ϵ -carboxymethoxycaproamido compound (IV), m.p. 163.5–164°; *Anal.* Calcd. for $C_{14}H_{18}N_2O_6S_2$: mol. wt., 342. Found: mol. wt. (Rast), 339. Thiolutin is regenerated by acetylation of II, affording an especially purified preparation of the antibiotic, referred to as the acetamido derivative (V),^{4,5} $\lambda_{\text{max}}^{\text{mu}}$ 250, ϵ 6300; $\lambda_{\text{max}}^{\text{nu}}$ 311, ϵ 5700; $\lambda_{\text{max}}^{\text{nu}}$ 388, ϵ 11,000. *Anal.* Calcd. for $C_8H_8N_2O_2S_2$: C, 42.09; H, 3.53; N, 12.28; S, 28.07. Found: C, 42.12; H, 3.77; N, 12.19; S, 27.72. Interestingly, the propionamido derivative (VI)^{4,5} is indistinguishable from aureothricin, $\lambda_{\text{max}}^{\text{mu}}$ 248, ϵ 6100; $\lambda_{\text{max}}^{\text{nu}}$ 312, ϵ 3900; $\lambda_{\text{max}}^{\text{nu}}$ 388, ϵ 11,000. *Anal.* Calcd. for $C_9H_{10}N_2O_2S_2$: C, 44.61; H, 4.16; N, 11.56; S, 26.40. Found: C, 44.97; H, 4.12; N, 11.54; S, 26.40.

Thiolutin and related compounds are inherently difficult to prepare free of solvent of crystallization. The earlier observed analytical data on Thiolutin and aureothricin coincidentally approximated both the tentative C_{13} formulations and the revised formulations. The high decomposition points and low solubility of these antibiotics in camphor make direct molecular weight determinations by the Rast method unreliable. Thiolutin and aureothricin possess strikingly similar antibiotic activity and ultraviolet absorption spectra. However, present paper chromatographic methods can resolve a mixture of Thiolutin and aureothricin as well as mixtures containing other homologous acylamido deriv-

(5) Thiolutin, aureothricin and the respective reacylated compounds V and VI decompose similarly at about 260 to 270° depending on the rate of heating.

atives of II. Furthermore, the infrared spectra (Nujol mulls) of closely related acyl derivatives of II exhibit differentiating absorption in the 12 to 14 micron region. Thiolutin is characterized by two similar, sharp bands at 12.1 and 12.5 microns, a dominantly, strong band at 13.45 microns and a partially resolved band at 13.6 microns. Aureothricin exhibits two similar, sharp bands at 12.2 and 12.6 microns, a partially resolved band at 13.45 microns and a dominantly strong band at 13.6 microns.

Structural studies are in progress and will be reported in subsequent publications.

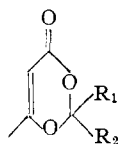
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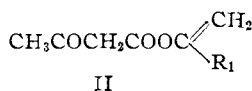
THE REACTION OF DIKETENE WITH KETONES Sir:

Although the preparation of diketene in acetone is a commercial process, its reactions with ketones to form compounds formulated as 2,2-disubstituted-4-methyl-6-keto-1,3-dioxenes, I, have escaped observation.

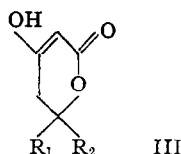


Ia, $R_1 = R_2 = \text{CH}_3$
Ib, $R_1 = \text{CH}_3, R_2 = \text{C}_6\text{H}_5$

The *p*-toluenesulfonic acid catalyzed reaction of diketene with acetone at 90° yields Ia (91%), b.p. 61–64° (5 mm.), n_D^{20} 1.464, d_4^{20} 1.079, $\lambda_{\text{max}}^{\text{EtOH}}$ 247.5 μ , $\log \epsilon$ 3.9; *Anal.* Calcd. for $\text{C}_7\text{H}_{10}\text{O}_3$: C, 59.14; H, 7.09; Found: C, 59.20; H, 7.15. The product from acetophenone, Ib, is crystalline, m.p. 93–94°, $\lambda_{\text{max}}^{\text{EtOH}}$ 247.5 μ , $\log \epsilon$ 3.86, $\lambda_{\text{max}}^{\text{isooctane}}$ 240 μ , $\log \epsilon$ 3.85; *Anal.* Calcd. for $\text{C}_{12}\text{H}_{12}\text{O}_3$: C, 70.57, H, 5.92; Found: C, 70.21; H, 5.81. These ketodioxenes are pleasant smelling liquids or crystalline solids easily handled in the absence of alkali. As many of their reactions parallel those of diketene they may conveniently be used in its place. Thus I reacts with alcohols, aniline and with mild alkali to yield acetoacetates, acetoacetanilide and dehydracetic acid, respectively. I does not react with carbonyl reagents and this and the ultraviolet spectra rule out structures II and III also considered.



II



III

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C-TERMINAL GROUPS OF TRYPSINOGEN, DFP-TRYPSIN AND CARBOXYPEPTIDASE¹

Sir:

A recent study of the effect of carboxypeptidase on chymotrypsinogen and DFP- α -chymotrypsin has led to the conclusion that the zymogen contains no C-terminal groups, in contrast to two such groups, leucine and tyrosine, in DFP- α -chymotrypsin.² In the present study, the same experimental technique was employed to investigate similarly the changes involved in the activation of trypsinogen and to test for the autolysis of carboxypeptidase.

Trypsinogen was crystallized in the presence of DFP³ and residual trypsin (less than 0.01%) was inactivated by the addition of a 2-fold excess of crystalline soybean trypsin inhibitor (Worthington). Twice recrystallized DFP-trypsin,³ containing less than 0.1% trypsin, was similarly inactivated. Seven times recrystallized carboxypeptidase was freed from residual amino acids (which previously were found to form a background on paper chromatograms²) by washing of the crystals with distilled water, and residual tryptic activity (less than 0.04%) was removed by the addition of a 100-fold molar excess of DFP to a solution of the enzyme in 10% LiCl.

Trypsinogen or DFP-trypsin was incubated with carboxypeptidase (substrate/enzyme mole ratio 11/1) at pH 7.5, 25°, and aliquots were removed at various time intervals up to 3 hours. Any free amino acids were absorbed onto and eluted from Dowex 50 resin^{2,4} and subjected to one-dimensional paper chromatography (butanol-acetic acid-water, 4:1:5 or phenol-*m*-cresol, 1:1). No amino acids whatsoever could be detected. Negative results were obtained also when a 2% solution of carboxypeptidase was similarly tested after it was allowed to autolyze up to 3 hours at 25°, pH 7.5.

These negative results suggest that (1) the protein substrates have no free C-terminal groups or (2) that these groups are not reactive toward carboxypeptidase either because they do not conform to the specificity requirements of the enzyme or because they are sterically inaccessible. The first explanation is rendered unlikely by the recent findings of Röver, Fabre and Desnuelle⁵ that trypsinogen and DFP-trypsin each contain one N-terminal group, valine and isoleucine, respectively, suggesting that these proteins are composed of at least one open polypeptide chain. The second interpretation receives support from the observation that following denaturation by acid, DFP-trypsin becomes reactive toward carboxypeptidase (substrate/en-

(1) DFP denotes di-isopropylfluorophosphate. This work was performed under contract No. Nonr-477-04 between the University of Washington and the Office of Naval Research, Department of the Navy, and was supported also by funds made available by the people of the State of Washington, Initiative 171.

(2) J. A. Gladner and H. Neurath, *Biochim. et Biophys. Acta*, **9**, 335 (1952), and unpublished experiments.

(3) L. W. Cunningham, Jr., F. Tietze, N. M. Green and H. Neurath, *Discussions of the Faraday Society*, in press; F. Tietze, in preparation.

(4) S. M. Partridge, *Nature*, **169**, 496 (1952); A. R. Thompson, *ibid.*, **169**, 495 (1952).

(5) M. Röver, C. Fabre and P. Desnuelle, *Biochim. et Biophys. Acta*, in press. We are indebted to Professor Desnuelle for informing us of these results prior to publication.