if the amount of reagent had been limited, the fully substituted derivative might appear in very small amount only.

With this method the major component of gramicidin-S has given the pattern shown in Fig. 1. It is therefore a di-acidic base. Weight and absorption measurements confirm the expectation that the band nearest the unchanged material is mono-substituted while that furthest removed is a bis-DNP-derivative. Deviation from Beer's law was slight. The unmistakable effect of substitution on the partition ratio is at once apparent. Such data further confirm other distribution data supporting the purity of the gramicidin-S fraction isolated.

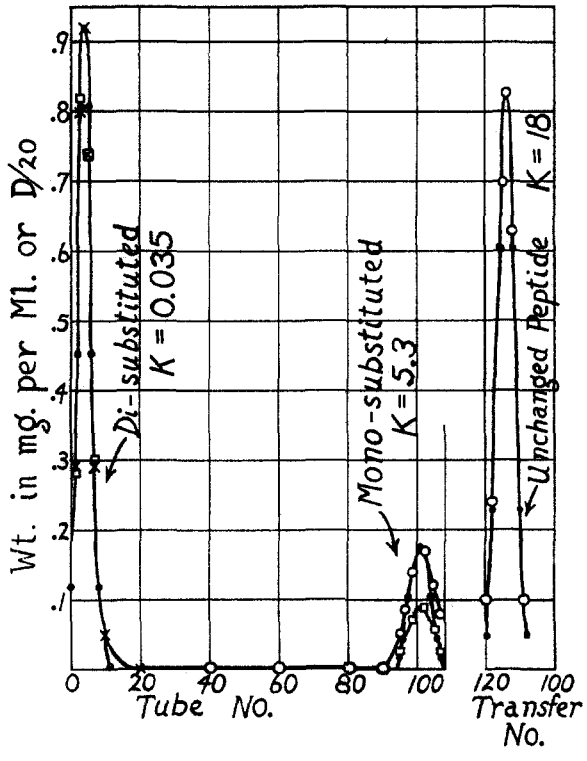



Fig. 1.—×—× = wt. per ml. in lower phase; ○—○ = wt. per ml. in upper phase; □—□ = D at 350 mμ; ●—● = theoretical curve. System = benzene, chloroform, methanol, 0.01 *N* HCl; volumes = 20, 10, 23, 7 ml., respectively; 120 transfers.

The molecular weight calculated approximates 1300. Quantitative amino acid residue determination[2] and X-ray studies[3] indicate another preparation of gramicidin-S to be either a pentapeptide, mol. wt. 571, or a decapeptide, mol. wt., 1142. This question is now definitely settled in favor of the latter.

Other peptides are being studied as above by partial esterification and by other reactions. Clear cut information has been obtained. An attempt is also being made to extend the method to the proteins.

FROM THE LABORATORIES OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
ALAN R. BATTERSBY[4]
LYMAN C. CRAIG

RECEIVED MARCH 14, 1951

(2) R. L. M. Synge, *Biochem. J.*, **39**, 363 (1945).
(3) D. C. Hodgkin, *Cold Spring Harbor Symposia*, **14**, 65 (1949).
(4) Commonwealth Fund Fellow 1950–1951.

## PURINE SYNTHESIS IN A PURINE-REQUIRING YEAST MUTANT

*Sir:*

An unusual case has been observed of a yeast mutant which will not grow unless supplied adenine or hypoxanthine, but nevertheless synthesizes purines as readily as does the wild type.

In these experiments, two haploid strains of *S. cerevisiae* were used—SC-7a, which grows on minimal medium containing no purines, and 285A, an ultraviolet mutant which is red in color and will grow only if adenine or hypoxanthine are added to the medium (guanine or 4-amino-5-imidazolecarboxamide will not support growth). The medium described by Reaume and Tatum[1] was used with the exception that casein hydrolysate was replaced by a mixture of *l*(−)-leucine, *l*(+)-lysine hydrochloride, *dl*-methionine and glutamine (50 mg./l.). Each yeast was grown for 48 hours with rapid aeration in 2 liters of this medium to which had been added 100 mg./l. of adenine sulfate and 40 mg./l. of glycine-1-$C^{14}$, the latter having previously been shown to be a precursor of yeast purines.[2] A smear of $10^5$ cells taken as the yeast (285A) was harvested showed no growth on a minimal medium-agar slant, indicating relative freedom from contamination and back mutation. Each yeast was fractionated by a modification of the Schmidt–Thannhauser[3] method to be published in detail elsewhere into acid soluble (ASN) purines, ribonucleic acid (RNA) purines, and desoxyribonucleic acid (DNA) purines. Adenine and guanine were obtained spectroscopically pure from each fraction by ion exchange chromatography.[4]

The data summarized in Table I indicate that the extent of incorporation of labeled glycine into the purines of the adenine-requiring mutant (285A) was of the same order of magnitude as in the wild type (SC-7a). In the case of the purines soluble in cold acid, the glycine incorporation was almost exactly the same in the two yeasts, while for the nucleic acids incorporation of $C^{14}$ in the mutant was one-half to two-thirds that of the wild type.

TABLE I

INCORPORATION OF GLYCINE-1-$C^{14}$ IN THE PURINES OF YEAST

| Yeast | SC-7a wild type | 285A adenine-requiring mutant |
|---|---|---|
| | Specific activity counts/min. of infinitely thick sample | |
| Glycine, carboxyl | $1.38 \times 10^6$ | $1.38 \times 10^6$ |
| ASN, Adenine | 3,490 | 3,205 |
| Guanine | 2,520 | 2,580 |
| RNA, Adenine | 1,499 | 1,004 |
| Guanine | 1,233 | 641 |
| DNA, Adenine | 1,850 | 1,323 |
| Guanine | 1,420 | 796 |

Experiments are in progress to elucidate the role of added purine in purine synthesis. At present we merely wish to point out that the production of a mutant which requires adenine does not necessarily imply a simple block in the pathway of

(1) S. E. Reaume and E. L. Tatum, *Arch. Biochem.*, **22**, 331 (1949).
(2) R. Abrams, E. Hammarsten and D. Shemin, *J. Biol. Chem.*, **173**, 429 (1948).
(3) G. Schmidt and S. J. Thannhauser, *ibid.*, **161**, 83 (1945).
(4) R. Abrams, *Arch. Biochem.*, **30**, 44 (1951).

adenine synthesis. The possibility exists that this type of phenomenon may occur in other biochemical mutations.

The initial stocks from which the yeasts used in these experiments were derived were kindly furnished by Dr. Carl C. Lindegren.

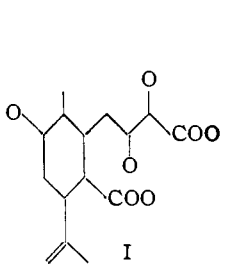
INSTITUTE OF RADIOBIOLOGY AND BIOPHYSICS
UNIVERSITY OF CHICAGO RICHARD ABRAMS
CHICAGO 37, ILLINOIS

RECEIVED MARCH 10, 1951

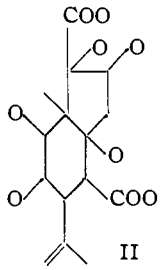
## THE SKELETON OF PICROTOXININ

*Sir:*

In order to account for the formation of picrotic acid and related substances, Robertson, *et al.*,[1] have proposed the partial carbon skeleton (I) for picrotoxinin, $C_{15}H_{16}O_6$, one of the two components of the amaroid picrotoxin. However picrotoxinin possesses two carbocyclic rings, *i.e.*, one more carbon-carbon bond must be drawn to complete the expression. *Evidence now obtained defines the location of*



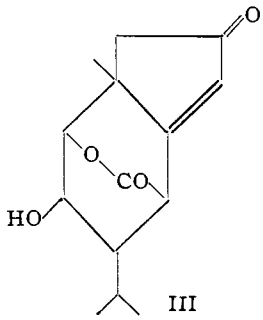
I



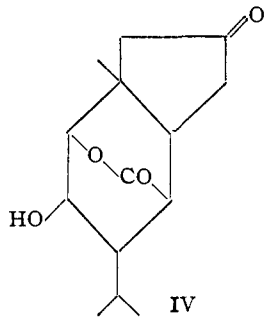
II

*the missing bond and requires that picrotoxinin be assigned the skeleton (II), one which lacks only five carbon atoms of a complete steroid nucleus.*

Dihydro-α-picrotoxininic acid[2] underwent smooth pyrolysis with loss of carbon dioxide and water to a new substance, designated picrotoxinide, $C_{14}H_{18}O_4$, not crystalline ($\lambda_{max.}$ 254 mμ, log $E$ 4.0; $\lambda_{max.}$ 2.95, 5.70, 5.84 and 6.20 μ) formulated as (III). Hydrogenation of (III) gave 90% of dihydropicrotoxinide (IV, m.p. 187°; calcd. for $C_{14}H_{20}O_4$: C, 66.64; H, 7.99. Found: C, 66.82; H, 8.11) which formed a 2,4-dinitrophenylhydrazone (m.p. 209° dec.; calcd. for $C_{20}H_{24}O_7N_4$: C, 55.53; H, 5.60.



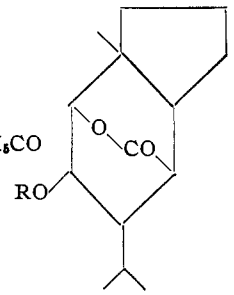
III

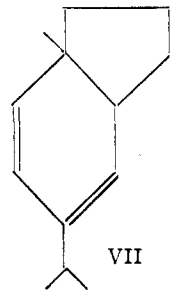


IV

Found: C, 55.42; H, 5.55) and a dibenzylidene derivative (m.p. 127–128°; calcd. for $C_{28}H_{28}O_4$: C, 78.48; H, 6.59. Found: C, 78.43; H, 6.74) strikingly similar in infrared and ultraviolet spectra to 2,5-dibenzylidenecyclopentanone.[3] The dihydroxyketo-acid from (IV) reacted with one mole of periodate. Dihydropicrotoxinide (IV) was converted to its ethylene mercaptal (m.p. 250°; calcd. for $C_{16}H_{24}O_3S_2$: C, 58.50; H, 7.36. Found: C, 58.52; H, 7.38) desulfurized with Raney nickel to tetrahydrodesoxypicrotoxinide (V; m. p. 162°; calcd. for $C_{14}H_{22}O_3$: C, 70.55; H, 9.31. Found: C, 70.65; H, 9.41; infrared $\lambda_{max.}$ 5.70 μ). The latter gave a benzoate (VI; m.p. 134°; calcd. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.56; H, 7.88) which underwent smooth pyrolysis to benzoic acid, carbon dioxide and picrotoxadiene (VII;

V, R = H
IV, R = $C_6H_5CO$





VII

b.p. 213°; $\lambda_{max}$ 256 mμ, log $E$ 3.6) characterized by its maleic anhydride adduct (m.p. 75°; calcd. for $C_{17}H_{22}O_3$: C, 74.42; H, 8.08. Found: C, 74.51; H, 8.31), the corresponding imide (m.p. 147–148°; $[\alpha]^{20}D$ −78° [chloroform, $c$ = 3.1]; calcd. for $C_{17}H_{23}O_2N$: C, 74.69; H, 8.48; N, 5.12. Found: C, 74.63; H, 8.55; N, 5.28) and the N-phenyl imide (m.p. 178°; $[\alpha]^{20}D$ −42° [chloroform, $c$ = 2.5]; calcd. for $C_{23}H_{27}O_2N$: C, 79.04; H, 7.79; N, 4.01; Found: C, 79.57; H, 7.80).

Synthetic *cis*-5-isopropyl-8-methylhydrin-4,6-diene (VII) was obtained by the action of isopropyl lithium on *cis*-8-methylhydrind-6-ene-5-one, (2,4-dinitrophenylhydrazone m.p. 138–139°; calcd. for $C_{16}H_{18}O_4N_4$: C, 58.16; H, 5.49. Found: C, 57.93; H, 5.41) prepared by an unambiguous route from *cis*-2-methyl 2-carboxycyclopentane-1-acetic acid.[4] Although the maleic anhydride adduct of the synthetic diene was not crystalline it gave an infrared spectrum identical with that of the natural adduct, and was converted to the crystalline imide (m.p. 158–159°, mixed m.p. with the natural imide 147–158°; found: C, 74.59; H, 8.36; N, 5.11) and the N-phenyl imide (m.p. 151–152°, mixed m.p. with the natural N-phenyl imide 151–175°; found: C, 79.04; H, 7.83; N, 3.88). Both imides gave infrared spectra identical with those from the corresponding natural derivatives; picrotoxadiene is clearly an optically active form of *cis*-5-isopropyl-8-methyl hydrin-4,6-diene (VII).

DEPARTMENT OF CHEMISTRY
HARVARD UNIVERSITY
CAMBRIDGE 38, MASS. HAROLD CONROY[5]

RECEIVED DECEMBER 19, 1950

(1) J. C. Harland and A. Robertson, *J. Chem. Soc.*, 937 (1939); D. Mercer, A. Robertson and R. S. Cahn, *ibid.*, 997 (1935).
(2) P. Horrmann, *Ber.*, **46**, 2793 (1913).
(3) D. Vorländer and K. Hobohm, *ibid.*, **29**, 1836 (1896).
(4) K. D. Errington and R. P. Linstead, *J. Chem. Soc.*, 666 (1938).
(5) National Institutes of Health Postdoctoral Fellow.

## THE STRUCTURE OF PICROTOXININ

*Sir:*

The skeleton (I) for picrotoxinin, $C_{15}H_{16}O_6$, has been proposed[1] to account for the formation of

(1) H. Conroy, THIS JOURNAL, **73**, 1889 (1951).