Reaction of β-Hydroxypropionic Acid and Sodium Cyanide.—The procedure in this experiment was identical with that described above for β-hydroxypropionitrile. β-Hydroxypropionic acid was first prepared by hydrolyzing the corresponding nitrile,[2] and the acid obtained was used without further purification. The amounts of β-hydroxypropionic acid, sodium cyanide and water used were 2.65 g., 8.62 g. and 15 ml., respectively. The succinic acid obtained in this case weighed 0.51 g. (14.9%) and gave m.p. of 181–182°.

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DEPARTMENT OF CHEMISTRY
YENCHING UNIVERSITY
PEKING, CHINA

RECEIVED OCTOBER 9, 1950

NEW COMPOUNDS

p-(2-Ethylhexoxy)-benzoic Acid

A mixture of 42 g. (0.2 mole) of 2-ethylhexyl bromide,[1] 28 g. (0.2 mole) of *p*-hydroxybenzoic acid, 22 g. (0.4 mole) of potassium hydroxide, 150 cc. of alcohol and 50 cc. of water was refluxed for 24 hours (two layers). In order to hydrolyze any ester which may still have been present, some 10% aqueous potassium hydroxide solution was added and the mass refluxed for another 30 minutes. Acidification gave an oily product which was isolated and distilled first under 35 mm. pressure (245°), then under 0.5 mm. (180°). The acid solidified easily and crystallized from a large amount of 80% formic acid in soft, white leaflets, which are converted at 60–61° into "liquid crystals," in analogy to the behavior of *p*-butoxybenzoic acid.[2]

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 72.0; H, 8.8; mol. wt., 250. Found: C, 72.4; H, 9.1; mol. wt., 255 (titration).

(1) Ch. Weizmann, E. Bergmann and L. Haskelberg, *Chem. and Ind.*, **56**, 1587 (1937).

(2) Bradfield and Jones, *J. Chem. Soc.*, 2660 (1929).

WEIZMANN INSTITUTE OF SCIENCE
REHOVOTH, ISRAEL S. PINCHAS

RECEIVED SEPTEMBER 1, 1950

2-Chlorophenyl-1-naphthylcarbinol[1]

2-Chlorophenyl-1-naphthylcarbinol.—A Grignard reagent was prepared from 11.6 g. of magnesium, 100 ml. of α-bromonaphthalene and 300 ml. of dry ether. When the reaction was complete, a solution of 55.3 g. of *o*-chlorobenzaldehyde in 300 ml. of dry ether was added dropwise with stirring. The mixture was stirred overnight at room temperature and then heated under reflux for one hour, cooled, and finally decomposed with 75 ml. of cold, 20% ammonium chloride solution. The ethereal solution was decanted from the hard residue and the residue was washed twice with ether. The combined ether solutions were washed with water, dried over "Drierite," and finally concentrated. The residue was fractionated under reduced pressure and the fraction distilling at 215–216° (4 mm.) was collected; yield 89.5 g. (85%).

A small portion of the above viscous oil was crystallized three times from dilute ethanol to give soft white needles; m.p. 96–97°.

Anal. Calcd. for $C_{17}H_{13}OCl$: C, 75.98; H, 4.88. Found: C, 76.16; H, 4.91.

(1) This compound is a previously unisolated intermediate in the synthesis of 1-(*o*-chlorobenzyl)-naphthalene by the method of Bradsher, THIS JOURNAL, **62**, 1077 (1940).

DEPARTMENT OF CHEMISTRY
VIRGINIA POLYTECHNIC INSTITUTE
BLACKSBURG, VIRGINIA FRANK A. VINGIELLO

RECEIVED NOVEMBER 13, 1950

COMMUNICATIONS TO THE EDITOR

THE MOLECULAR WEIGHT DETERMINATION OF POLYPEPTIDES

Sir:

In the course of our attempts to purify and characterize certain of the higher polypeptides by countercurrent distribution it has become apparent that any change affecting a carboxyl or amino group produces a striking effect on the partition ratio of the peptide. Accordingly, the derivatives representing different stages of substitution which result from incomplete reaction can be separated readily. Since with unchanged peptide remaining, the band occurring in the distribution pattern nearest the unchanged substance would be the monosubstituted derivative, a general approach to the problem of molecular weights is suggested. Molecular weight determination by analysis for substituting groups has long been a standard procedure but a decision as to the numbers of groups involved has often been equivocal.

For instance, if a polypeptide with one free $-NH_2$ is treated with sufficient 2,4-dinitrofluorobenzene[1] so that only a fraction of it is converted, distribution of the resulting mixture in a suitable system gives two bands, one of the unchanged polypeptide and a yellow band of the DNP derivative. Determination of the distribution pattern by weight and by absorption at 350 mμ now permits calculation of the molecular weight, provided Beer's law holds. The value ϵ of δ-DNP-ornithine hydrochloride (16,250) can be used as the basis of calculation.

If the peptide contains two amino groups, four bands in the pattern are possible. One band containing the disubstituted product will be furthest removed from that containing the unchanged material. Two possible intermediate mono-substituted products would be expected to have very similar partition ratios and could form overlapping bands unless a very high number of transfers had been applied. Across any one colored band the ratio of weight to absorption should be, and in our experience thus far has been, constant.

With three amino groups in the molecule, two families of overlapping bands and one of the completely substituted peptide could occur. However,

(1) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

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.

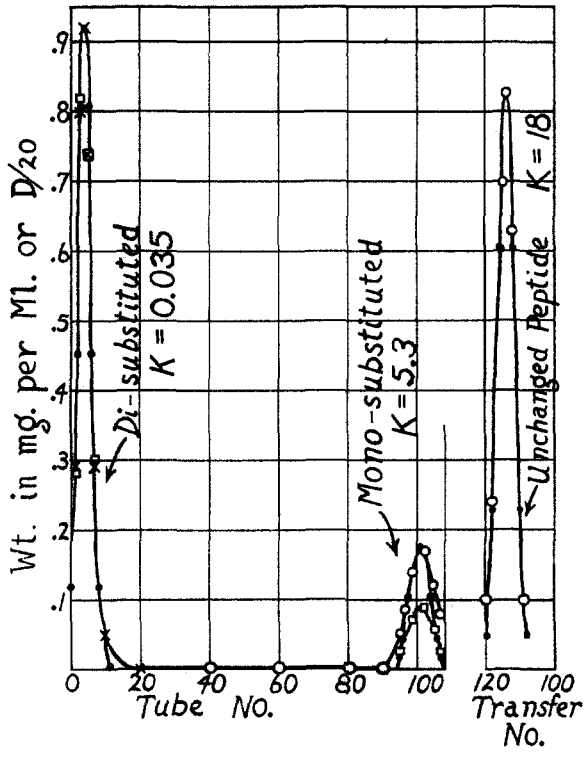

Fig. 1.—×—× = wt. per ml. in lower phase; ○—○ = wt. per ml. in upper phase; □—□ = D at 350 mμ; ●—● = theoretical curve. System = benzene, chloroform, methanol, 0.01 *N* HCl; volumes = 20, 10, 23, 7 ml., respectively; 120 transfers.

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	1.38×10^6	1.38×10^6
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).