We found in these solutions the presence of the following ions: $Mo_7O_{24}^{-6}$ ("paramolybdate of Delafontaine"), $Mo_6O_{20}^{-4}$ ("trimolybdate"), $Mo_6O_{20}H^{-3}$ ("tetramolybdate"). The paramolybdic ion of Rosenheim ($Mo_6O_{24}H^{-5}$) does not exist in detectable amount in these solutions.

Further details will be published in *J. Chim. Phys.* or may be found in the author's thesis, "Contribution à l'étude de l'électrode à quinhydrone: application à la détermination des isopolyanions molybdiques."

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Total Syntheses of N^{α}-[1-(2-Acetamido-3-*O*-D-glucosyl)-D-propionyl-L-alanyl-D-α- and γ-glutamyl]-L-lysyl-D-alanyl-D-alanine, and Identity of the γ-Glutamyl Isomer with the Glycopeptide of a Bacterial Cell Wall Precursor

Sir:

Accumulation of uridine nucleotides in a *Staphylococcus aureus* was observed[1] to occur when its growth was inhibited by penicillin. On the basis of degradation[2,3] and enzymatic synthesis[4] the principal compound, containing the amino sugar muramic acid [2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose],[5,6] was assigned the structure, uridine-5′-pyrophosphoryl-*N*-acetylmuramyl-L-alanyl-D-glutamyl-L-lysyl-D-alanyl-D-alanine. Further characterization of the nucleotide from penicillin-treated cells[7] and from enzymatic synthesis[4d] provided evidence for the N^{α}-γ-glutamyllysyl peptide linkage. The glycopeptide formed by mild acid hydrolysis[1c,4b] of the uridine nucleotide may then be completely formulated as II.

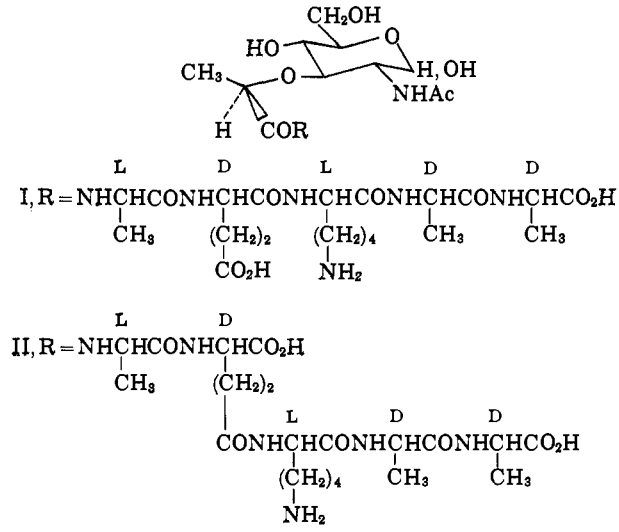
We wish to record total synthesis of N^{α}-[1-(2-acetamido-3-*O*-D-glucosyl)-D-propionyl-L-alanyl-D-α- and γ-glutamyl]-L-lysyl-D-alanyl-D-alanine (I and II), and to report that the γ-glutamyl isomer II is identical with the glycopeptide of a bacterial cell wall precursor, as shown by two-dimensional paper chromatography.

H-N^{ϵ}-Z-L-Lys-OH[8,9] (Na salt) and *t*-butylazidoformate[10] in refluxing aqueous *t*-butyl alcohol gave N^{α}-*t*-BOC-N^{ϵ}-Z-L-Lys-OH as a colorless viscous oil which, esterified[11] with *p*-nitrophenol and *N*,*N*′-dicyclohexylcarbodiimide, gave N^{α}-*t*-BOC-N^{ϵ}-Z-L-Lys-ONP[12] (III), m.p. 83–85°, $[\alpha]^{24}D$ −23.6° (*c* 2.0, DMF). Activated ester III with H-D-Ala-D-Ala-ONBZ[13] gave N^{α}-*t*-BOC-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ (IV), m.p. 124–125°, $[\alpha]^{24}D$ +9.5° (*c* 2.0, DMF). Selective removal (HCl + HOAc[14,15]) of the *t*-BOC group from tripeptide IV yielded H-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ·HCl·H_2O (V), m.p. 158–159°, $[\alpha]^{25}D$ +37.8° (*c* 2.8, DMF).

t-BOC-(γ-OBZ)-D-Glu-OH, obtained as a colorless viscous oil from γ-benzyl D-glutamate,[16] was esterified with *p*-nitrophenol to yield *t*-BOC-(γ-OBZ)-D-Glu-ONP (VI), m.p. 120–121°, $[\alpha]^{25}D$ 32.3° (*c* 2, DMF). Condensation of activated ester VI with tripeptide derivative V in DMF, with addition of one equivalent of triethylamine, gave N^{α}-[*t*-BOC-(γ-OBV)-D-α-Glu]-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ·0.25H_2O (VII), m.p. 145–147°, $[\alpha]^{25}D$ +13.8° (*c* 2.1, DMF). Removal (HCl + HOAc) of the *t*-BOC group from tetrapeptide derivative VII afforded N^{α}-[H-(γ-OBZ)-D-α-Glu]-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ·HCl·0.5H_2O (VIII), m.p. 123–124° dec., $[\alpha]^{24}D$ −9.1° (*c* 2, DMF).

t-BOC-L-Ala-ONP (IX), m.p. 82–83, $[\alpha]^{25}D$ −60.5° (*c* 2, ethanol), obtained by esterification of *t*-BOC-L-Ala-OH,[15] was condensed with tetrapeptide derivative VIII to yield N^{α}-[*t*-BOC-L-Ala-(γ-OBZ)-D-α-Glu]-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ·0.5H_2O (X), m.p. 181–182° dec., $[\alpha]^{25}D$ +22.5° (*c* 2, DMF). The latter pentapeptide derivative gave, with HCl + HOAc, N^{α}-[H-L-Ala-(γ-OBZ)-D-α-Glu]-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ·HCl·H_2O (XI), m.p. 194–195° dec., $[\alpha]^{25}D$ +19.6°.

Benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside[17] (XII) was condensed in acetonitrile with pentapeptide XI (with addition of one equivalent of triethylamine) by means of *N*-ethyl-5-phenylisoxazolium-3′-sulfonate[18] to afford



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the D-α-glutamyl isomer of the fully protected *N*-acetylmuramyl pentapeptide, N^{α}-[1-(2-acetamido-1-*O*-benzyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-D-glucopyranosyl)-D-propionyl-L-Ala-(γ-OBZ)-D-α-Glu]-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ·H_2O (XIII), m.p. 211–214° dec., $[\alpha]^{25}D$ +58.5° (*c* 1, DMF). Hydrogenolysis (H_2/Pd black/10% Pd–C/85% HOAc) of XIII, and purification of the resulting product by means of column chromatography on Celite diatomaceous earth, yielded with 4.5 holdback volumes (H.B.V.) of butanol–acetic acid–water (6:1:4) the D-α-glutamyl isomer I of the *N*-acetylmuramyl pentapeptide as a colorless hygroscopic amorphous solid which sinters at 105°, m.p. 145–148° dec., $[\alpha]^{25}D$ +33.6° (*c* 1.3, water). *Anal.* Calcd. for $C_{31}H_{53}N_7O_{15}\cdot 2.5H_2O$: C, 46.1; H, 7.23; N, 12.1. Found: C, 46.1; H, 7.18; N, 12.2.

α-Benzyl D-glutamate,[19] *t*-butylazidoformate, and sodium carbonate in refluxing aqueous dioxane gave *t*-BOC-D-Glu-OBZ as a colorless oil which was esterified with *p*-nitrophenol to yield *t*-BOC-(γ-ONP)-D-Glu-OBZ (XIV), m.p. 101–102°, $[\alpha]^{25}D$ +22.2° (*c* 2.5, DMF). Activated ester XIV was condensed with tripeptide derivative V to yield N^{α}-[*t*-BOC-(α-OBZ)-D-γ-Glu]-N^{ϵ}-Z-L-Oys-D-Ala-D-Ala-ONBZ (XV), m.p. 174–175°, $[\alpha]^{25}D$ +16.7° (*c* 2.5, DMF). Removal of the *t*-BOC group from XV (HCl + HOAc) afforded N^{α}-[H-(α-OBZ)-D-γ-Glu]-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ·HCl·$0.5H_2O$ (XVI), m.p. 141–142° dec., $[\alpha]^{25}D$ +5.5° (*c* 2, DMF).

Condensation of activated alanine ester IX with tetrapeptide ester XVI yielded N^{α}-[*t*-BOC-L-Ala-(α-OBZ)-D-γ-Glu]-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ (XVII), m.p. 187–188° dec., $[\alpha]^{25}D$ +11.0° (*c* 2, DMF). Removal (HCl + HOAc) of the *t*-BOC group from XVII afforded N^{α}-[H-Ala-(α-OBZ)-D-γ-Glu]-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ·HCl·H_2O (XVIII), m.p. 153–154° dec., $[\alpha]^{23}D$ +24.2° (*c* 2, DMF).

The base from pentapeptide salt XVIII was condensed with protected muramic acid XII by means of *N*-ethyl-5-phenylisoxazolium-3′-sulfonate in acetonitrile to yield the D-γ-glutamyl isomer of the fully protected muramyl pentapeptide, N^{α}-[1-(2-acetamido-1-*O*-benzyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-D-glucopyranosyl)-D-propionyl-L-Ala-(α-OBZ)-D-γ-Glu]-N^{ϵ}-Z-L-Lys-D-Ala-D-Ala-ONBZ (XIX), m.p. 215–218° dec., $[\alpha]^{25}D$ +46.9° (*c* 1, acetic acid). Hydrogenolysis of XIX and chromatography as for XIII afforded (with 7.9 H.B.V.) the D-γ-glutamyl isomer II of the *N*-acetylmuramyl pentapeptide as a colorless, hygroscopic, amorphous solid, m.p. 148–150° dec., $[\alpha]^{25}D$ +14.0° (*c* 0.9, water). *Anal.* Calcd. for $C_{31}H_{53}N_7O_{15}\cdot H_2O$: C, 47.6; H, 7.09; N, 12.5. Found: C, 47.5; H, 7.25; N, 12.5.

Two-dimensional paper chromatography was employed to compare isomers I and II with enzymatically synthesized glycopeptide (kindly carried out by Anderson and Strominger[20]).[20a] A single radioactive and ninhydrin-positive spot was obtained when γ-glutamyl isomer II and *N*-acetylmuramyl-L-alanyl-D-glutamyl-^{14}C-L-lysyl-D-alanyl-D-alanine (XX) (obtained[21] by the action of venom phosphodiesterase and alkaline phosphatase on uridine-5′-pyrophosphoryl-*N*-acetylmuramyl-L-alanyl-D-glutamyl-^{14}-C-L-lysyl-D-alanyl-D-alanine[22]) were cochromatographed on paper with isobutyric acid:0.1 *M* ammonium hydroxide (5:3) (solvent A) used for the first dimension and pyridine:water (4:1) (solvent B) for the second. The α-glutamyl isomer I was more mobile than the labeled glycopeptide with the ratio of mobilities of $\gamma/\alpha = 0.83$ in solvent A and 0.97 in solvent B.

N^{α}-(L-Alanyl-D-γ-glutamyl)-L-lysyl-D-alanyl-D-alanine (obtained by hydrogenolysis of fully protected pentapeptide XVII) was cochromatographed on paper with L-alanyl-D-glutamyl-^{14}C-L-lysyl-D-alanyl-D-alanine (obtained[21] by the action of acetylmuramyl-L-alanine amidase[23] on the ^{14}C-labeled *N*-acetylmuramyl pentapeptide XX), and was clearly differentiated from N^{α}-(L-alanyl-D-α-glutamyl)-L-lysyl-D-alanyl-D-alanine (obtained by hydrogenolysis of the corresponding protected pentapeptide). The ratio of mobilities of γ/α was 0.77 in solvent A and 0.60 in solvent B.

Acknowledgment.—We wish to thank Drs. J. S. Anderson and J. L. Strominger[20] for paper chromatographic comparisons of their enzymatically synthesized compounds with I, II, and the corresponding pentapeptides, Mr. L. Brancone and staff for microanalyses, Mr. W. Fulmor and staff for optical rotation measurements, Mr. J. W. Marsico for paper chromatography, and Mr. C. Pidacks for column chromatography.

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(20) Department of Pharmacology, University of Wisconsin Medical School, Madison, Wis.

(20a) NOTE ADDED IN PROOF.—Identity of the acetylmuramyl pentapeptide and the pentapeptide from enzymatically prepared nucleotide with the D-γ-glutamyl isomer II and N^{α}-(L-alanyl-D-γ-glutamyl)-L-lysyl-D-alanyl-D-alanine was also established by means of a paper electrophoresis on Whatman 3MM paper in 0.18 *M* pyridine acetate buffer of pH 4.1 at a potential gradient of 16 volts/cm. at 0° for 5 hr.

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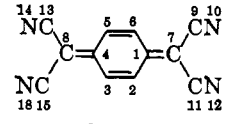
C^{13} Hyperfine Splittings in the 7,7,8,8-Tetracyanoquinodimethane Anion Radical

Sir:

We have measured the C^{13} hyperfine splittings of the 7,7,8,8-tetracyanoquinodimethane (TCNQ) anion radical in which C^{13} was substituted in positions 1 (4) and 9 (11, 13, 15). Our results (Table I) show (1) that the



TCNQ

C^{13} splittings calculated from simple Hückel–LCAO or McLachlan[1] theory and the σ–π parameters of Fraenkel, *et al.*,[2,3] are not in very good agreement with experiment and (2) that previous assignments[4] based

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