

rotational barrier<sup>14</sup> or by causing temperature-dependent differential chemical shifts of the sort discussed by Buckingham, *et al.*<sup>15</sup>

However, for aromatic compounds in which internal rotation is absent or strongly hindered, the variation of the chemical shift with temperature is much smaller than that of biphenyl-4,4'-*d*<sub>2</sub> or 4,4'-dimethylbiphenyl, the solvent being carbon disulfide in all these cases.<sup>16</sup>

TABLE I

Solvent	Substituent	Concentration, mg./ml.	$\nu_0(\delta)$ , c.p.s. at 60 Mc.p.s.	
			Obsd.	Calcd.
C <sub>6</sub> H <sub>12</sub>	D	200	10.5	10.3
C <sub>6</sub> H <sub>11</sub> CH <sub>3</sub>	D	33	10.8	10.3
CH <sub>2</sub> Cl <sub>2</sub>	D	33	9.3	10.3
(CHCl <sub>2</sub> ) <sub>2</sub>	D	200	9.3	10.3
Diglyme	D	200	9.1	10.3
(CH <sub>3</sub> ) <sub>2</sub> CO	D	33	10.8	10.3
CH <sub>3</sub> CN	D	33	10.7	10.3
CS <sub>2</sub>	D	33	7.2	10.3
CH <sub>2</sub> Cl <sub>2</sub>	CH <sub>3</sub>	13	14.2	
CS <sub>2</sub>	CH <sub>3</sub>	13	12.8	
(CHCl <sub>2</sub> ) <sub>2</sub>	F	200	21.5	27.6
C <sub>6</sub> H <sub>6</sub>	Cl		10.0 <sup>a</sup>	7.2
CH <sub>2</sub> Cl <sub>2</sub>	Cl		6 <sup>a</sup>	7.2
(CHCl <sub>2</sub> ) <sub>2</sub>	Cl	200	>3.8 <i>ca.</i>	7.2
Diglyme	Cl	200	8.9	7.2
(CHCl <sub>2</sub> ) <sub>2</sub>	Br	200	9.2	8.4
Diglyme	Br	200	>3.8 <i>ca.</i>	8.4
(CHCl <sub>2</sub> ) <sub>2</sub>	I	200	29.9	28.8
(CHCl <sub>2</sub> ) <sub>2</sub>	NO <sub>2</sub>	200	32.4	34.2
Diglyme	NO <sub>2</sub>	200	22.9	34.2

<sup>a</sup> D. M. Grant, R. C. Hirst, and H. S. Gutowsky, *J. Chem. Phys.*, **38**, 470 (1963).

Equations 3a and 3b may be expanded to third order in  $x$  and  $y$  and fitted by least squares to the observed temperature variation of  $\nu_0(\delta)$ . Values obtained for  $V_2$  are  $4.4 \pm 0.5 \times 10^2$  cal./mole,  $7 \pm 1 \times 10^2$  cal./mole, and  $11.0 \pm 2.5 \times 10^2$  cal./mole for biphenyl in methylcyclohexane, chloroform-carbon tetrachloride, and carbon disulfide, respectively, and limits for  $V_4$  of  $\pm 100$  cal./mole. These values are consistent with a potential barrier which is relatively small and which has a minimum close to a dihedral angle of  $\pi/2$ .<sup>17</sup>

(14) A. K. Colter and L. C. Clemens have found solvent effects in the racemization rate of 1,1'-binaphthyl [*J. Phys. Chem.*, **68**, 651 (1964)].

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(16) The aromatic line positions of the following compounds show no change (to within experimental error,  $\pm 0.2$  to  $\pm 0.4$  c.p.s., depending on line width) over the indicated temperature range: toluene, *o*-xylene, *m*-xylene, *p*-xylene (38 to  $-86^\circ$ ), phenanthrene (33 to  $-56^\circ$ ), 4,6-dimethyl-dibenzothiophene ( $-31$  to  $36^\circ$ ). For 2-methylbiphenyl and 2,2'-dimethylbiphenyl-4,4'-*d*<sub>2</sub>, the changes in differential (*ortho-meta*) chemical shift are *ca.*  $1.8 \pm 0.8$  c.p.s. and  $1.8$  to  $2.5$  c.p.s., respectively, over the temperature range  $44$  to  $-90^\circ$ ; the extrapolated change for biphenyl-4,4'-*d*<sub>2</sub> over this same range is  $4.0$  c.p.s.

(17) F. Adrian [*J. Chem. Phys.*, **28**, 608 (1958)] and I. Fischer-Hjalmars [*Tetrahedron*, **19**, 1805 (1963)] have both calculated potential energy curves which possess shallow minima at about  $40^\circ$  rather than  $90^\circ$ , agreeing with the electron diffraction results of ref. 4.

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### Study of Isopolymolybdates in Aqueous Solution with the Aid of the Quinhydrone Electrode

Sir:

The quinhydrone electrode and its salt error have been studied, after Biilmann,<sup>1</sup> mainly by American

authors such as Cullen,<sup>2</sup> Morgan, Lammert, and Campbell,<sup>3</sup> Corran and Lewis,<sup>4</sup> La Mer and Baker,<sup>5</sup> Hovorka and Dearing,<sup>6</sup> Gabbard,<sup>7</sup> Harned and Wright,<sup>8</sup> and Hayes and Lietzke.<sup>9</sup> We feel it, therefore, worthwhile to present in *J. Am. Chem. Soc.* a brief summary of the main conclusions we were led to, while studying the formation of isopolymolybdates with the aid of the quinhydrone electrode.

(1) If we define the salt error of the quinhydrone electrode as  $\Delta E = -RT/2F \ln f_h/f_q$  ( $f_h$  and  $f_q$  being the activity factors of hydroquinone and quinone, respectively), we conclude, in opposition with the results obtained by Gabbard,<sup>7</sup> that, in a given salt solution, for instance  $3 M$  NaCl, the salt error  $\Delta E$  is independent of the pH. This has been proved between pH 1.00 and 8.25, for well buffered solutions, by measuring the e.m.f. of the cell

Pt; H<sub>2</sub> (1 atm.), buffer + NaCl (3 M), quinhydrone; Au

whose constant value (after correction for the small salt error due to the buffer) was found to be 0.69140 abs. v.  $\pm 0.2$  mv. at  $25^\circ$ .

(2) We have shown that in poorly buffered mediums the "acidifying effect  $\Delta pH$ " due to the ionization of hydroquinone is given by

$$\log \Delta pH = \log dpH/dx + \log S/C + pH - pK'$$

with the following notations: the pH is that of the solution under test, the  $pK'$  is that of hydroquinone (considered in a first approximation as a weak monobasic acid) in the given salt solution, and  $S$  is the concentration of hydroquinone (equal to the solubility of quinhydrone) in the given medium.  $C$  is the concentration of the buffer and  $dpH/dx$  the reciprocal of the buffer capacity.

(3) The standard potential of the quinhydrone electrode was found to be 0.69972 abs. v.  $\pm 0.03$  mv. at  $25^\circ$ .

(4) A receipt for recrystallization of quinhydrone has been indicated, and a method to verify its stoichiometry with an accuracy of 0.02% has been described.

(5) Different distributing effects on the potential of the quinhydrone electrode have been studied, *e.g.*, reaction with glycine buffer, oxidation through molybdates, and drifting of quinone vapors by inert gases like nitrogen or argon.

(6) Recrystallization of NaCl has been described; to avoid the formation of traces of NaOH, wet recrystallized NaCl must be dried at a temperature not higher than  $45^\circ$ .

(7) The study of the molybdates was made by means of progressive displacement of the molybdic acid from Na<sub>2</sub>MoO<sub>4</sub> solutions, with HCl, and measuring the pH. All solutions were  $3 M$  in respect to NaCl and the concentration of Na<sub>2</sub>MoO<sub>4</sub> varied from  $M/2$  to  $M/3200$ .

The interpretation was made with the Bye, Souchay, and Lefebvre<sup>10</sup> method of the "potentiometric surface."

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- (8) H. S. Harned and D. D. Wright, *ibid.*, **55**, 4849 (1933).
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We found in these solutions the presence of the following ions:  $\text{Mo}_7\text{O}_{24}^{-6}$  ("paramolybdate of Delafontaine"),  $\text{Mo}_6\text{O}_{20}^{-4}$  ("trimolybdate"),  $\text{Mo}_6\text{O}_{20}\text{H}^{-3}$  ("tetramolybdate"). The paramolybdic ion of Rosenheim ( $\text{Mo}_6\text{O}_{24}\text{H}^{-5}$ ) 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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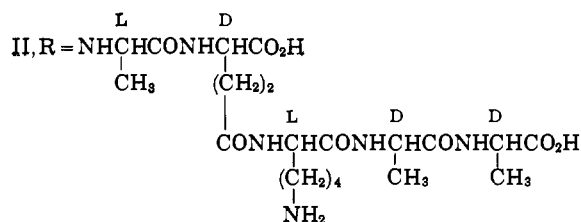
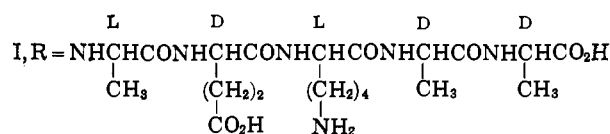
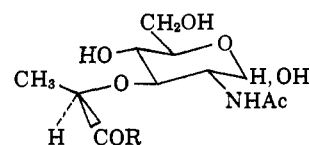
**Total Syntheses of  $N^\alpha$ -[1-(2-Acetamido-3-O-D-glucosyl)-D-propionyl-L-alanyl-D- $\alpha$ - and  $\gamma$ -glutamyl]-L-lysyl-D-alanyl-D-alanine, and Identity of the  $\gamma$ -Glutamyl Isomer with the Glycopeptide of a Bacterial Cell Wall Precursor**

Sir:

Accumulation of uridine nucleotides in a *Staphylococcus aureus* was observed<sup>1</sup> to occur when its growth was inhibited by penicillin. On the basis of degradation<sup>2,3</sup> and enzymatic synthesis<sup>4</sup> the principal compound, containing the amino sugar muramic acid [2-amino-3-O-(D-1-carboxyethyl)-2-deoxy-D-glucose],<sup>5,6</sup> 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<sup>7</sup> and from enzymatic synthesis<sup>4d</sup> provided evidence for the  $N^\alpha$ - $\gamma$ -glutamyllysyl peptide linkage. The glycopeptide formed by mild acid hydrolysis<sup>1c,4b</sup> of the uridine nucleotide may then be completely formulated as II.

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

H- $N^\epsilon$ -Z-L-Lys-OH<sup>8,9</sup> (Na salt) and *t*-butylazidiformate<sup>10</sup> in refluxing aqueous *t*-butyl alcohol gave  $N^\alpha$ -*t*-BOC- $N^\epsilon$ -Z-L-Lys-OH as a colorless viscous oil which, esterified<sup>11</sup> with *p*-nitrophenol and *N,N'*-dicyclohexylcar-



bodiimide, gave  $N^\alpha$ -*t*-BOC- $N^\epsilon$ -Z-L-Lys-ONP<sup>12</sup> (III), m.p. 83–85°,  $[\alpha]^{24\text{D}} - 23.6^\circ$  (*c* 2.0, DMF). Condensation of activated ester III with H-D-Ala-D-Ala-ONBZ<sup>13</sup> gave  $N^\alpha$ -*t*-BOC- $N^\epsilon$ -Z-L-Lys-D-Ala-D-Ala-ONBZ (IV), m.p. 124–125°,  $[\alpha]^{24\text{D}} + 9.5^\circ$  (*c* 2.0, DMF). Selective removal (HCl + HOAc<sup>14,15</sup>) of the *t*-BOC group from tripeptide IV yielded H- $N^\epsilon$ -Z-L-Lys-D-Ala-D-Ala-ONBZ·HCl·H<sub>2</sub>O (V), m.p. 158–159°,  $[\alpha]^{25\text{D}} + 37.8^\circ$  (*c* 2.8, DMF).

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

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

Benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(D-1-carboxyethyl)-2-deoxy- $\alpha$ -D-glucopyranoside<sup>17</sup> (XII) was condensed in acetonitrile with pentapeptide XI (with addition of one equivalent of triethylamine) by means of *N*-ethyl-5-phenylisoxazolium-3'-sulfonate<sup>18</sup> to afford

(12) Unless otherwise noted, all compounds were obtained as colorless crystals; satisfactory analyses were obtained for these compounds.

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(9) The following abbreviations are employed: Ala = alanine, Glu = glutamic acid, Lys = lysine, *t*-BOC = *t*-butoxycarbonyl, BZ = benzyl, NBZ = *p*-nitrobenzyl, NP = *p*-nitrophenyl, Z = benzyloxycarbonyl, DMF = *N,N*-dimethylformamide.

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