rotational barrier[14] or by causing temperature-dependent differential chemical shifts of the sort discussed by Buckingham, *et al.*[15]

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_2 or 4,4′-dimethylbiphenyl, the solvent being carbon disulfide in all these cases.[16]

TABLE I

Solvent	Substituent	Concentration, mg./ml.	$\nu_0\langle\delta\rangle$, c.p.s. at 60 Mc.p.s. Obsd.	Calcd.
C_6H_{12}	D	200	10.5	10.3
$C_6H_{11}CH_3$	D	33	10.8	10.3
CH_2Cl_2	D	33	9.3	10.3
$(CHCl_2)_2$	D	200	9.3	10.3
Diglyme	D	200	9.1	10.3
$(CH_3)_2CO$	D	33	10.8	10.3
CH_3CN	D	33	10.7	10.3
CS_2	D	33	7 2	10.3
CH_2Cl_2	CH_3	13	14.2	
CS_2	CH_3	13	12.8	
$(CHCl_2)_2$	F	200	21.5	27.6
C_6H_6	Cl		10.0[a]	7.2
CH_2Cl_2	Cl		6[a]	7.2
$(CHCl_2)_2$	Cl	200	>3.8 *ca.*	7.2
Diglyme	Cl	200	8.9	7.2
$(CHCl_2)_2$	Br	200	9.2	8.4
Diglyme	Br	200	>3.8 *ca.*	8.4
$(CHCl_2)_2$	I	200	29.9	28.8
$(CHCl_2)_2$	NO_2	200	32.4	34.2
Diglyme	NO_2	200	22.9	34.2

[a] 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 $\nu_0\langle\delta\rangle$. 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 ± 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 $\pi/2$.[17]

(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)].

(15) A. D. Buckingham, T. Schaefer, and W. G. Schneider, *J. Chem. Phys.*, **32**, 1227 (1960).

(16) The aromatic line positions of the following compounds show no change (to within experimental error, ±0.2 to ±0.4 c.p.s., depending on line width) over the indicated temperature range: toluene, *o*-xylene, *m*-xylene, *p*-xylene (38 to −86°), phenanthrene (33 to −56°), 4,6-dimethyldibenzothiophene (−31 to 36°). For 2-methylbiphenyl and 2,2′-dimethylbiphenyl-4,4′-d_2, the changes in differential (*ortho–meta*) chemical shift are *ca.* 1.8 ± 0.8 c.p.s. and 1.8 to 2.5 c.p.s., respectively, over the temperature range 44 to −90°; the extrapolated change for biphenyl-4,4′-d_2 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° rather than 90°, 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,[1] mainly by American authors such as Cullen,[2] Morgan, Lammert, and Campbell,[3] Corran and Lewis,[4] La Mer and Baker,[5] Hovorka and Dearing,[6] Gabbard,[7] Harned and Wright,[8] and Hayes and Lietzke.[9] 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,[7] that, in a given salt solution, for instance 3 M NaCl, the salt error ΔE 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_2 (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. ± 0.2 mv. at 25°.

(2) We have shown that in poorly buffered mediums the "acidifying effect ΔpH" 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. ± 0.03 mv. at 25°.

(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°.

(7) The study of the molybdates was made by means of progressive displacement of the molybdic acid from Na_2MoO_4 solutions, with HCl, and measuring the pH. All solutions were 3 M in respect to NaCl and the concentration of Na_2MoO_4 varied from $M/2$ to $M/3200$.

The interpretation was made with the Bye, Souchay, and Lefebvre[10] method of the "potentiometric surface."

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(6) F. Hovorka and W. C. Dearing, *ibid.*, **57**, 446 (1935).
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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). Condensation of 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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