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.

(19) H. Sachs and E. Brand, *J. Am. Chem. Soc.*, **75**, 4610 (1953).

(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.

(21) J. S. Anderson and J. L. Strominger,[20] private communication.

(22) P. M. Meadow, J. S. Anderson, and J. L. Strominger, *Biochem. Biophys. Res. Commun.*, **14**, 382 (1964).

(23) J. M. Ghuysen and J. L. Strominger, *Biochemistry*, **2**, 1110 (1963).

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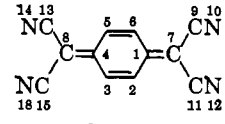
C^{13} Hyperfine Splittings in the 7,7,8,8-Tetracyanoquinodimethane Anion Radical

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

(1) A. D. McLachlan, *Mol. Phys.*, **3**, 233 (1960).

(2) M. Karplus and G. K. Fraenkel, *J. Chem. Phys.*, **35**, 1312 (1961).

(3) P. H. Rieger and G. K. Fraenkel, *ibid.*, **37**, 2795 (1963).

(4) P. H. H. Fischer and C. A. McDowell, *J. Am. Chem. Soc.*, **85**, 2694 (1963).

on the above theories and the hyperfine splittings arising from C^{13} in natural abundance are in error.

TABLE I

Li^+TCNQ^- SPLITTING CONSTANTS[a]

Position[b] or splitting constant	Calculated,[c] oersted— Hückel	McLachlan	Exptl., oersted— Ref. 4	C^{13} subsd.[d]
A_{N14}			1.02	1.009 (±0.005)
A_H			1.44	1.415 (±0.004)
$C^{13}(1)$	−3.11	−5.02	4.40	1.52 (±0.04)
$C^{13}(2)$	+0.48	+0.79	0.62	
$C^{13}(7)$	+5.23	+8.49	7.18	
$C^{13}(9)$	−7.17	−8.44	6.38	7.06 (±0.04)

[a] g = 2.00263 ± 0.00005, 10^{-4} M in tetrahydrofuran. [b] See TCNQ structure for position numbering. [c] See ref. 3 and 4. [d] g value and hyperfine splittings obtained by comparison in a dual cavity with a 10^{-4} M solution in THF of lithium tetracyanoethylenide whose g = 2.00270 ± 0.00005 and A_N = 1.574.

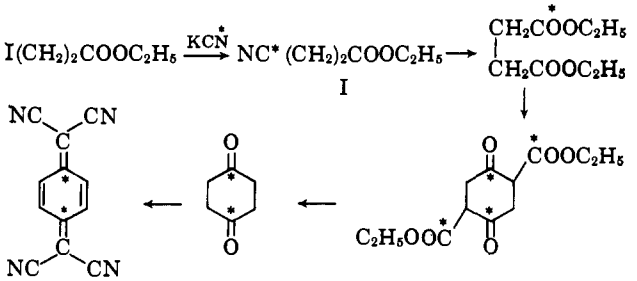
We have observed in the unlabeled $TCNQ^-$ spectrum two sets of low intensity lines. One set arises from C^{13} at position 9. The other with one-half the signal intensity of that arising from position 9 has a splitting of 4.6 oersted. If the latter is due to C^{13} splitting, it must arise from the methylene carbons (*i.e.*, 7, 8). We have been unable to observe any other resonances in the unlabeled $TCNQ^-$ spectrum.

Table I shows that the C^{13} splitting constants for positions 1 and 9 differ from those previously assigned[4] and from those calculated from theory. The difference is even more pronounced if the value of 4.6 oersted can be associated with the C^{13} splitting by position 7. One concludes that either (perhaps both) the current theory of C^{13} hyperfine splitting or spin-density calculation is inadequate and requires revision.

The two C^{13} enriched TCNQ anion radicals were synthesized as follows. For TCNQ enriched by C^{13} in position 1 (4) (Scheme I), ethyl β-iodopropionate was treated with potassium cyanide-C^{13} to give β-ethoxycarbonyl propionitrile-1-C^{13} (I). Treatment of

SCHEME I

SYNTHESIS OF TCNQ-1(4)-C^{13}



I with ethanolic hydrogen chloride gave diethyl succinate-1-C^{13} which was treated with sodium ethoxide in ether to give 2,5-bis(ethoxycarbonyl)-1,4-cyclohexanedione-C^{13} which was hydrolytically decarboxylated to 1,4-cyclohexanedione-1(4)-C^{13}. The C^{13}-labeled cyclohexanedione was converted to TCNQ-1(4)-C^{13} and LiTCNQ-1(4)-C^{13} by the usual procedures.[5,6] Mass spectral analysis of CO_2 resulting from combustion of the labeled TCNQ gave a value for C^{13}/C^{12} corresponding to ~5% C^{13} in each of positions 1 and 4, in excellent agreement with the expected value as well as with the relative e.s.r. line amplitudes.

TCNQ enriched by C^{13} in position 9 (11, 13, 15) was prepared by the reaction of potassium cyanide-C^{13} with TCNQ to give K^+TCNQ^- 9(11, 13, 15)-C^{13} which was then oxidized to the correspondingly labeled TCNQ.[7] Mass spectral analysis again gave a value for C^{13}/C^{12} corresponding to ~5% C^{13} in each of the positions, in good agreement with relative e.s.r. line amplitudes.

(5) D. S. Acker and W. R. Hertler, *J. Am. Chem. Soc.*, **84**, 3370 (1962).

(6) L. R. Melby, R. J. Harder, W. R. Hertler, W. Mahler, R. E. Benson, and W. E. Mochel, *ibid.*, **84**, 3374 (1962).

(7) This method of labeling is analogous to that used in the preparation of cyano-labeled tetracyanoethylene-C^{14} [O. W. Webster, W. Mahler, and R. E. Benson, *ibid.*, **84**, 3678 (1962)].

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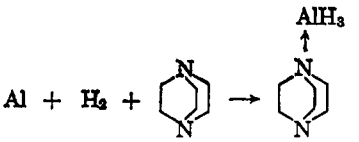
The Direct Synthesis of Amine Alanes

Sir:

In previous papers we have reported the use of the Al–H_2 reducing system for the synthesis of $NaAlH_4$[1,2] and amine boranes.[3] Now we wish to report the first successful direct synthesis of an amine alane, triethylenediamine alane, by reaction of aluminum and hydrogen in the presence of triethylenediamine at moderate temperature and pressure.



In a typical experiment 30 g. of triethylenediamine was dissolved in 100 ml. of tetrahydrofuran. To this solution was added approximately 6 g. of activated aluminum powder.[2] The resulting mixture was heated for 6 hr. at 70° and 5000 p.s.i. hydrogen pressure. A light gray solid was isolated by filtration and analyzed for aluminum, hydrogen, and nitrogen. The nitrogen analysis was performed by potentiometric titration of the solution obtained on hydrolysis of the product, after removal of the $Al(OH)_3$. *Anal.* Calcd. for $C_6H_{12}N_2 \cdot AlH_3$: Al, 19.0; N, 19.7; H, 21.2 mmoles/g. Found: Al, 21.8; N, 19.1; H, 22.4 mmoles/g.

Deuterolysis of the product showed that 92.4% of the evolved gas was DH and 7.6% was D_2, indicating a low concentration of unreacted aluminum. The product is thermally stable to >200° and reacts violently with water. It is insoluble in the common organic solvents tested and for this reason no molecular weight determination has been made. However, it is speculated that the product is not monomeric, because of the difunctional nature of the amine. The ready formation

(1) E. C. Ashby (to Ethyl Corp.), French Patent 1,235,680 (May 30, 1960).

(2) E. C. Ashby, *Chem. Ind.* (London), 208 (1962).

(3) E. C. Ashby and W. E. Foster, *J. Am. Chem. Soc.*, **84**, 3407 (1962).