which additional tetracyclic diene **2** (contaminated with some $\Delta^{17,17a}$ isomer) could be isolated by preparative tlc. A fraction with a lower R_f than that containing **2** appeared, by nmr and ir analysis, to contain mainly tricyclic material.

Treatment of the crude tetracyclic diene **2** with excess osmium tetroxide in pyridine for 48 hr at 24° followed by cleavage of the bisosmate with hydrogen sulfide in dimethyl sulfoxide gave a solid tetraol which, without purification, was treated with excess lead tetraacetate in tetrahydrofuran at 0° for 15 min. The crude solid product was then stirred with 2.5% aqueous potassium hydroxide for 13 hr at 74°.[7] Preparative tlc afforded, in 29% over-all yield, crystalline *dl*-16,17-dehydroprogesterone, mp 182–184° after crystallization from benzene–hexane (*Anal.* Found: C, 80.5; H, 9.0). The nmr, solution ir, uv, and mass spectra of the synthetic material were identical with the corresponding spectra of authentic naturally derived material.

We wish to emphasize that, in view of the preliminary nature of this work, considerable improvement in the yields cited above may be anticipated as the study continues.

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(7) The ring-D closure procedure of W. F. Johns (*J. Am. Chem. Soc.*, 80, 6456 (1958)) was employed. See also G. Stork, K. N. Khastgir, and A. J. Solo, *ibid.*, 80, 6457 (1958).

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A Solid-Phase Synthesis of [Lysine]-vasopressin through a Crystalline Protected Nonapeptide Intermediate[1,2]

Sir:

We wish to report the synthesis of the crystalline protected nonapeptide, S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide (I),[3] by the solid-phase method developed by Merrifield.[4] Highly active [lysine]-vasopressin was obtained from I with excellent recovery. Recent reports about solid-phase syntheses of deamino-oxytocin[5] and of oxytocin[6,7] *via* protected nonapeptide intermediates prompt us to communicate our studies.

The preparation of I was carried out according to the general procedure described by Marshall and Merrifield[8] for solid-phase syntheses, where a cycle for the incorporation of each amino acid into the growing peptide chain involved acidolytic cleavage of the N-protecting *t*-butyloxycarbonyl (Boc) group, treatment with triethylamine, and peptide bond formation, with repeated washing operations in between. The starting *t*-butyloxycarbonylglycyl resins contained 0.22–0.27 mmol of glycine/g[9] in several preparations. Dicyclohexylcarbodiimide[10] was used as the coupling reagent for Boc-Pro, Boc-Cys(Bzl), Boc-Phe, Boc-Tyr, and Tos-Cys(Bzl). The lysine, asparagine, and glutamine moieties were introduced into the growing peptide chain by the *p*-nitrophenyl ester method[11] using Boc-Lys(Tos)-ONp,[12] Boc-Asp(NH_2)-ONp,[13] and Boc-Glu(NH_2)-ONp,[14] respectively. All protected amino acid derivatives[15] were repeatedly recrystallized until the known criteria of purity compared favorably.

In order to evaluate the progress during the first three cycles, crystalline benzyloxycarbonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide (II)[3] was isolated by branching off a part of the resin (1.5 g) after the second cycle and introducing Z-Pro. Subsequent ammonolysis[16] for 70 hr in a sealed flask at room temperature containing absolute ethanol (35 ml) which had been saturated at 0° with anhydrous ammonia afforded an oil which was crystallized from ethyl acetate to give colorless prisms, 270 mg (98%[17]),[18] mp 185–186°, $[\alpha]^{23}D$ −32.5° (*c* 1, dimethylformamide); lit.[3] mp 184–185°, $[\alpha]^{20.5}D$ −33.0° (*c* 1, dimethylformamide).

Ammonolysis of the peptide resin (3.7 g, corresponding to 2.4 g of Boc-Gly-resin of 0.27 Gly equivalents) after the completion of all nine cycles was carried out as described above except that a mixture (1:1) of absolute ethanol and dimethylformamide was used. Evaporation of the solvents afforded a crude oil. It was dissolved in dimethylformamide. Addition of water precipitated an amorphous solid (1.0 g) which was treated

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(2) Abbreviations follow the rules of the IUPAC–IUB Commission on Biochemical Nomenclature, in *Biochemistry*, 5, 1445, 2485 (1966); 6, 362 (1967); *J. Biol. Chem.*, 241, 2491 (1966).

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(17) All yields are based on the glycine content of the starting Boc-Gly-resin.

(18) This compares favorably with a yield of 40% of Z-Pro-Leu-Gly-NH_2, obtained by ammonolysis with liquid ammonia during a solid phase synthesis of oxytocin by Beyerman, *et al.*[6]

repeatedly with boiling methanol to constant weight. The remaining solid (0.42 g, 42%[17]) was crystallized from dimethylformamide containing 1% formic acid to give I as colorless needles, 260 mg (26%[17]), mp 229–231°, $[\alpha]^{23}D$ −24.2° (*c* 2, dimethylformamide); lit.[3] mp 228–230°, $[\alpha]^{20.5}D$ −24.0° (*c* 2, dimethylformamide). A mixture melting point with authentic material[3,19] showed no depression. A sample for analysis was recrystallized from dimethylformamide–formic acid (99:1) with 95% recovery, mp 230–231°, $[\alpha]^{23}D$ −24.7° (*c* 2, dimethylformamide); lit.[3] mp 231–232°, $[\alpha]^{22}D$ −24.5° (*c* 2, dimethylformamide). *Anal.* Calcd for $C_{74}H_{91}O_{16}N_{13}S_4$ (mol wt 1546.8): C, 57.5; H, 5.93; N, 11.8. Found: C, 57.2; H, 5.80; N, 11.5. The infrared spectra of I and of authentic material were identical.

Amino acid analysis[20] of I gave the expected ratios of the constituent amino acids. Similar correct ratios were obtained from amino acid analyses of all fractions prior to crystallization, and even of the crude oil. This indicated that data obtained from amino acid analyses might be misleading if used by themselves as criteria of purity for peptides prepared by the solid phase method.

Conversion of I to [lysine]-vasopressin was carried out as described previously.[3] Thus treatment of I (50 mg) with sodium in liquid ammonia to remove all protecting groups, oxidative cyclization, desalting, and lyophilization gave a powder (37 mg, 95%) exhibiting approximately 250 units of pressor activity[21] per mg. Amino acid analysis of a sample which had been oxidized by performic acid[22] gave: $CySO_3H$, 1.90; Asp, 1.01; Glu, 1.00; Pro, 0.97; Gly, 1.00; Tyr, 0.95; Phe, 1.00; Lys, 1.03; NH_3, 3.01. Further purification[3] by ion-exchange chromatography afforded with 70% recovery [lysine]-vasopressin possessing a pressor activity of 308 ± 14 units/mg.

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Substituent Effects in the M − 42 Rearrangement of *n*-Butylbenzenes[1]

Sir:

The electron impact induced decomposition of *n*-butylbenzene requiring hydrogen transfer with benzylic cleavage yields an M − 42 fragment ion and competes with formal, simple benzylic cleavage for the major fraction of the fragment total ion current.[2–4] Earlier observations render doubtful the participation of a route which involves hydrogen transfer to benzylic carbon and support the McLafferty rearrangement.[3,5] Our data (Table I) also support these conclusions and argue against rearranged structures similar to those postulated to account for the behavior of substituted phenetoles, which have nearly identical Z/Z_0 values for *meta* and *para* isomers with the same substituent.[6,7]

Table I. Substituent Effects[a] on the Abundance of M − 42 Ions from $[XC_6H_4CH_2CH_2CH_2CH_3]\cdot^+$

X	Z/Z_0, 12 eV	Z/Z_0, 70 eV
H	1.00	1.00
p-NH_2	0.06	0.09
m-NH_2	0.47	1.15
p-OH	0.06	0.07
m-OH	0.84	1.15
p-OCH_3	0.03	0.05
m-OCH_3	0.76	1.15
p-F	0.15	0.19
m-F	1.05	1.24
p-I	0.09	0.05
m-I	0.52	0.27
p-CH_3	0.17	0.17
m-CH_3	0.73	0.81
p-$COOCH_3$	0.73	0.56
m-$COOCH_3$	0.90	0.73
p-COOH	1.02	0.64
m-COOH	1.22	0.94
p-CN	0.70[b]	1.75
m-CN	1.45	1.88
p-NO_2	1.05	0.94
m-NO_2	1.48	1.15

[a] $Z = [XC_7H_7]\cdot^+/[XC_6H_4CH_2CH_2CH_2CH_3]\cdot^+$. [b] Value at 14 eV is 1.32.

In the McLafferty rearrangement there are basically two processes which might be influenced by substituents: hydrogen transfer to the benzene ring and carbon–carbon bond cleavage.[8] If the carbon–carbon bond breaking here parallels the behavior observed in the M − 43 decomposition,[2] all *meta* substituents would be expected to exert a substantial effect in reducing Z/Z_0 ratios to similar small values, whereas *para* electron-donating groups (−X) should tend to enhance the relative rates of decomposition and give comparatively larger Z/Z_0 values. That these are not observed can be attributed to a considerable substituent effect on the transfer site or, without excluding this possibility, to a

(1) Financial assistance from the Research Corporation and from Eli Lilly and Company is gratefully acknowledged. The AEI MS-9 mass spectrometer used was purchased with funds made available by National Science Foundation Grant GP 3672.

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(3) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1967, pp 73–85 and references cited therein.

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(5) A third alternative which involves hydrogen transfer to the ring at the point of alkyl substitution would not be expected to show reduced transfer with substitution of one *ortho* methyl group (reduced ~50%) or two *ortho* methyl groups (zero transfer). See ref 3, p 82.

(6) F. W. McLafferty, M. M. Bursey, and S. M. Kimball, *J. Am. Chem. Soc.*, **88**, 5022 (1966).

(7) For a review of substituent effects in mass spectrometry see M. M. Bursey, *Org. Mass Spec.*, **1**, 31 (1968).

(8) The site, γ,[4] of carbon–hydrogen cleavage is probably too remote to be affected by the aromatic ring and its substituents.