Table I. Insecticidal *N*-Carbalkoxy-*O*-alkylalkanamidoximes, $R_1C(=NOR_2)NHCO_2R_3$

Compd No.	R_1	R_2	R_3	Bp (mm), °C	Refractive index, n^tD (*t*)	% yield	Composition[d]
1	Et	Et	Et	50–52 (0.025)	1.4560 (20)	43	$C_8H_{16}N_2O_3$
2	Et	Et	Me	61–62 (0.02)	1.4525 (22)	31	$C_7H_{14}N_2O_3$
3	Me	$PhCH_2$	Me	106.5–107.5 (0.03)	1.5283 (23)	57	$C_{11}H_{14}N_2O_3$[a]
4	Me	Et	Me	44–45 (0.04)	1.4550 (23)	39	$C_6H_{12}N_2O_3$
5	Et	$PhCH_2$	Et	118–120 (0.06)	1.5189 (22)	45	$C_{13}H_{18}N_2O_3$[b]
6	Et	$PhCH_2$	Me	114–115 (0.05)	1.5248 (23)	36	$C_{12}H_{16}N_2O_3$[c]
7	Me	Me	Me	31.5–32.5 (0.03)	1.4557 (24)	16	$C_5H_{10}N_2O_3$

[a] H: calcd, 6.30; found, 5.79. [b] N: calcd, 11.20; found, 11.60. [c] C: calcd, 61.01; found, 62.44. [d] *Anal.* C, H, N. In cases where elemental analyses were poor, the nmr spectra were consistent with those expected for the compds.

Table II. Insecticidal Activity of *N*-Carbalkoxy-*O*-alkylalkanamidoximes

Compd No.	Insects killed/total tested					
	C. vicina	*D. melanogaster*	*A. verbasci*	*O. surinamenis*	*T. molitor*	*B. germania*
1	17/20	6/8	0/5	5/15	0/5	0/5
2	13/20	6/7	2/5	0/11	0/5	0/5
3	9/16	8/9	3/6	4/11	0/5	0/5
4	7/14	5/5	2/6	1/6	0/5	0/5
5	3/23	0/5	0/5	1/6	0/5	0/5
6	0/25	0/6	3/5	0/5	1/5	0/5
7	7/12	0/4	0/5	0/5	0/5	0/5

(10 cm diam) and 3–5 insects were introduced and observed for 1 hr. After 1 hr of contact the organisms were transferred to clean petri dishes or in the case of blowflies to 250-ml erlenmeyer flasks which contd food and H_2O. Controls were run with filter paper that had been sprayed with Et_2O and air-dried.

It can be seen (Table II) that the blowfly and fruit fly are most sensitive to the test compds. The blowflies became agitated on contact (within 5 min) with all of the test compounds. After 15 or 20 min they showed convulsions alternating with paralysis. When death occurred it was during the first hour while the insects were still in contact with the test compd. Those that survived the hour of contact always recovered. Aside from the fact that they showed decreased activity when in contact with the test compds, fruit flys showed no unusual behavior. Cockroaches and mealworms appeared to be completely unaffected.

The fact that insects recovered if not killed during contact indicates that the test compds have typical carbamate activity since carbamate activity (cholinesterase inhibition) is rapidly reversed when the insecticide is withheld due to dissociation of the enzyme-substrate complex.‡ Further, the selectivity demonstrated by the test compds is also characteristic of carbamates,[4] and as expected the heavily sclerotized *Coleoptera* and *Orthoptera* were not affected.‡ The LD_{50} of the most active compd **1**, was determined in mice by injecting 5% solns in tragacanth ip. At 750 mg/kg and 1000 mg/kg 2 out of 5 mice died.

Experimental Section

The prepn of the most active compd in the series of *N*-carbethoxy-*O*-ethylpropanamidoxime, **1** ($R_1 = R_2 = R_3 = Et$), illustrates the general method. 2-Ethoxyiminobutyramide (14.4 g, 0.1 mole) was dissolved in 90 ml of commercial abs EtOH, and 2 equiv of NaOEt in commercial abs EtOH was added. The addn of 1 equiv of Br_2 with stirring caused a white ppt (NaBr) to form. After 10 min of heating on the steam bath the mixt was cooled and neutralized with AcOH. The NaBr was sepd by filtration, and the EtOH was distd from the filtrate. The residual oil was taken up in Et_2O, washed (H_2O), and dried ($MgSO_4$). Distn of Et_2O and fractionation of the residue (60-mm Vigreaux column) produced a 43% yield of material which boiled at 50–52° (0.025 torr): $n^{20}D$ 1.4560; ir (film) 1648 (C=N) and 1725 cm^{-1} (monosubstituted urethane); nmr (in ppm, internal TMS) ($CDCl_3$) 1.18 (3 overlapping triplets, 9 H, CH_3CH_2), 2.64 (q, 2 H, $N=CCH_2$), 4.09 (m, 4 H, OCH_2CH_3), 7.74 (s, 1 H, NH, exchangeable with D_2O). *Anal.* ($C_8H_{16}N_2O_3$) N, calcd, 14.89. Found, 14.32. In spite of the poor N anal. the nmr was consistent with that expected for the compd.

2-Ethoxyiminobutyramide is a new compd prepd by an established procedure[5] in 77% yield: mp 44–45°. *Anal.* ($C_6H_{12}N_2O_2$) C; H: calcd, 8.33. Found, 7.66.

2-Ethoxyiminopropanamide was prepd by an established procedure[5] in 72% yield: mp 64–65°. *Anal.* ($C_5H_{10}N_2O_2$) C; H: calcd, 7.61. Found, 8.14.

References

(1) M. H. J. Weiden and H. M. Moorefield, *World Rev. Pest Cont.*, **3**, 102 (1964).
(2) E. Jeffreys, *Ber.*, **30**, 898 (1897).
(3) D. A. Coviello, *J. Med. Chem.*, **7**, 121 (1964).
(4) J. E. Casida, *Annu. Rev. Entomol.*, **8**, 39 (1963).
(5) D. W. Woolley, J. W. Hershey, and H. A. Jodlowsky, *J. Org. Chem.*, **28**, 2012 (1963).

‡A. W. A. Brown, University of Western Ontario, private communication.

Inhibition Studies on Antibody to Poly(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-*1*-^{14}C Ethyl Ester. Synthesis and Immunochemical Properties of Poly(L-alanyl-L-glutamyl-L-alanylglycyl)glycine-*1*-^{14}C Ethyl Ester

Brian J. Johnson,* and Charles Cheng† and Nora Tsang‡

Department of Biochemistry, and Department of Microbiology and Immunology, University of Alabama Medical School, Birmingham, Alabama 35233.

Received July 9, 1971

A recent investigation of the immunochemical properties of poly(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-*1*-^{14}C ethyl ester[1,2] has shown that the polypeptide is antigenic in rabbits.[3] It has been shown that the tyrosyl residue is an important moiety in enhancing antibody formation. To investigate this point further, it was considered that the substitution of the tyrosyl residue with the nonaromatic alanyl residue may affect the immunochemical properties of the molecule. To this end we wish to report the immunochemical properties of poly(L-alanyl-L-glutamyl-L-alanylglycyl)-glycine-*1*-^{14}C.

Chemistry. The polymerizing unit Ala-γ-*tert*-Bu-Glu-Ala-Gly pentachlorophenyl ester hydrochloride (**5**) and the necessary intermediates for its preparation were synthesized as detailed in the Experimental Section. The polymerization was performed at a reagent concn of 100 mmoles/l. in the

†Ph.D. Thesis, Tufts University, Medford, Mass.
‡Senior Research Thesis, Tufts University, Medford, Mass.

presence of a preformed monomer since this has been shown to produce linear high molecular weight polypeptides.[1,2,4-8] Following this established procedure the polymer, poly(Ala-γ-*tert*-Bu-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester was prepared; from which the protecting *tert*-Bu groups were removed by the use of 90% F_3CCO_2H to yield poly(Ala-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester (**1**). After extensive dialysis, the polymer was purified and fractionated by passage through calibrated columns of Sephadex G-100[9] and Corning CPG-10-240 glass granules. By this means the mol wt of the polypeptide was found to be at least 1×10^5.

Immunochemistry. Two rabbits were immunized with **1** using the same protocol as that previously described.[3] To aliquots of the sera obtained from each rabbit was added incremental amounts of the polypeptide **1**. No precipitin reaction was observed. A number of polymers have been tested for the ability to inhibit the homologous reaction of poly(Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester antisera. Of this $poly(Glu)_n$, $(Tyr\text{-}Glu\text{-}Ala\text{-}Gly)_1\text{-}poly(Lys)_n$ and $(Tyr\text{-}Glu\text{-}Ala\text{-}Gly)_3Gly$ have not exhibited inhibition capacity.§ However, poly(Glu-Tyr-Ala-Gly)Gly-*l*-^{14}C Et ester[4] has been shown to be a mild inhibitor causing 50% inhibition of precipitation in the presence of 6000 μg of this polypeptide.

The possibility that the peptide **1** could be a hapten for the antigen poly(Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester was also investigated. Incremental amounts of the polypeptide **1** were added to aliquots of antisera to poly(Tyr-Glu-Ala-Gly)-Gly-*l*-^{14}C Et ester containing the equivalence point of the antigen. Inhibition of the precipitin reaction was observed, as shown in Figure 1, such that 50% inhibition of precipitation was obtained in the presence of 750 μg of the polypeptide **1**.

Conclusions

It is known that a number of antigens, poly(Phe-Glu-Ala-Gly)gly-*l*-^{14}C Et ester,[10] poly(Tyr-Glu-Val-Gly)Gly-*l*-^{14}C Et ester,[11] and poly(OMe-Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester[10] cross-react with poly(Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester antisera. It is also known that poly(Tyr-Glu-Gly-Gly)Gly-*l*-^{14}C Et ester[5] and poly(Glu-Tyr-Ala-Gly)Gly-*l*-^{14}C Et ester[4] do not cross-react with poly(Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester antisera. The polypeptide **1** is unique in that at present it has not yet been found to be antigenic and does not cross-react but rather acts as a potent inhibitor of the homologous reaction of poly(Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester antisera. As such, it provides information about the antigen specificity and conformation of the poly(Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester molecule. It would appear that the overall conformation of the polymer **1** is similar to that of the antigen since it competes well for coverage of part of the antigenic determinants of the antibodies to poly(Tyr-Glu-Ala-Gly)-Gly-*l*-^{14}C Et ester. Thus it has been concluded that the overall conformation of the molecule is maintained by the sequence $(X\text{-}Glu\text{-}Ala\text{-}Gly)_n$. It would appear that the role of the tyrosyl residue of the antigen while not necessary to maintain the conformation is necessary for antigen specificity.

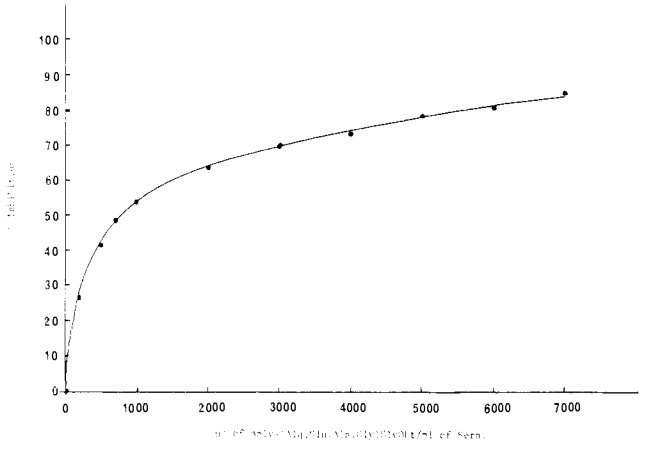


Figure 1.

Experimental Section

Melting points were taken with a Mel-Temp apparatus and are uncor.

Z-Ala-γ-*tert*-Bu-Glu-Ala-Gly Me Ester (2).[#] To a soln of 5.4 g (14.2 mmoles) of γ-*tert*-Bu-Glu-Ala-Gly Me ester · HCl and 1.5 g (14.9 mmoles) of Et_3N in 200 ml of CH_2Cl_2 was added 6.0 g (12.6 mmoles) of Z-Ala pentachlorophenyl ester. The mixt was stirred overnight at room temp and concd, and the product was dissolved in EtOAc, washed with 10% citric acid soln and H_2O, and then dried (Na_2SO_4) and concd *in vacuo* to give a solid. This material was chromatogd on a column of Silicar CC-7 using $CHCl_3$–EtOAc (1:1) as eluent, to give the fully blocked tetrapeptide; crystn from EtOAc–hexane yielded 4.3 g (62%): mp 191°, $[\alpha]^{24}D$ −8.1° (*c* 3.27, DMF). *Anal.* ($C_{26}H_{38}N_4O_9$) C, H, N.

Z-Ala-γ-*tert*-Bu-Glu-Ala-Gly (3). To a soln of 4.3 g (7.8 mmoles) of Z-Ala-γ-*tert*-Glu-Ala-Gly Me ester in 350 ml of MeOH was added 7.82 ml of 1 *N* KOH and the soln was stirred for 90 min at room temp and then concd under reduced pressure. The residue was flooded with H_2O, acidified with 10% citric acid soln, and extd into EtOAc. The EtOAc soln was dried (Na_2SO_4) and concd under reduced pressure to give the tetrapeptide free acid, crystn from EtOAc–hexane yielded 3.5 g (83.5%): mp 183, $[\alpha]^{24}D$ −5.8° (*c* 2.5, DMF). *Anal.* ($C_{25}H_{36}N_4O_9$) C, H, N.

Z-Ala-γ-*tert*-Bu-Glu-Ala-Gly Pentachlorophenyl Ester (4). To a soln of 3.5 g (6.5 mmoles) of the tetrapeptide free acid **3** in 300 ml of CH_2Cl_2 were added 1.9 g (7.0 mmoles) of pentachlorophenol and 3.2 g (7.5 mmoles) of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate. The mixt was shaken for 2 days at room temp, and then concd under reduced pressure to give a solid. This material was washed with H_2O and crystd from MeOH to yield 2.5 g (48.8%): mp 205°, $[\alpha]^{24}D$ −8.7° (*c* 1.67, DMF). *Anal.* ($C_{31}H_{35}Cl_5N_4O_9$) C, H, N.

Ala-γ-*tert*-Bu-Glu-Ala-Gly Pentachlorophenyl Ester · HCl (5). A fine suspension of 3.3 g (4.2 mmoles) of the tetrapeptide active ester 4 and 0.8 g of 10% Pd/C in 200 ml of MeOH was treated with 0.153 g (4.2 mmoles) of dry HCl in MeOH, and the suspension was hydrogenated for 2 hr. The reaction mixt was filtered, and the filtrate was concd. The residue was crystd from MeOH–Et_2O to give 1.5 g (52%): mp 225°, $[\alpha]^{25}D$ + 1 (*c* 0.9, DMF). *Anal.* ($C_{23}H_{30}Cl_6N_4O_7$) C, H, N.

Poly(Ala-Glu-Ala-Gly)Gly-*l*-^{14}C Et Ester (1). To a soln of 0.78 g (7.63 mmoles) of Et_3N and 0.6 mg of Gly-*l*-^{14}C Et ester · HCl in 5 ml of DMSO was added a soln of 1.5 g (2.18 mmoles) of the polymerizing unit **5** in 17 ml of DMSO. The mixt was shaken for 1 week and then centrifuged to yield the product which was washed with three 35-ml portions of H_2O, three 35-ml portions of MeOH, and three 35-ml portions of Et_2O and dried to give 0.254 g (30.3%) of the blocked polymer. This material was treated with 30 ml of 90% F_3CCO_2H and stirred for 50 min, and then concd under reduced pressure to yield the crude polypeptide **1**. This material was suspended in 20 ml of H_2O, and dissolved by addn of 1 *N* NaOH to pH 7.5. The soln was dialyzed against distd H_2O overnight and acidified with 6 *N* HCl to pH 2.5. The pptd polypeptide **1** was collected by centrifugation and then lyophilized to yield 0.1 g (14%). *Anal.* ($C_{13}H_{20}N_4O_6$) C, H, N.

Molecular Weight Determination. Calibrated columns of Sephadex G-100 (2.5 × 38.5 cm) and of Corning CPG 10-240 glass granules (2 × 35 cm) were employed for the mol wt determination. Using 0.15 *M* NaCl as eluent, 4 mg of the Na salt of poly(Ala-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester was passed through each of these columns. The polypeptide was eluted from each column in a vol equiv to that corresponding to a mol wt of at least 1×10^5.

Immunochemical Results. Two rabbits were treated at weekly

§ Nora Tsang, unpublished results.

[#] Z = benzyloxycarbonyl.

intervals with 500 μg of poly(Ala-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester 1. The first 2 weeks they were injected intradermally using complete Freunds adjuvant as suspension medium and the 3rd week they were injected sc. The injection on the 4th week was done iv using buffered saline. Bleedings were conducted on the following week and the serum from each animal was not found to give a precipitin reaction with up to 100 μg of polymer 1.

Inhibition Studies. To 1-ml aliquots of rabbit antisera to poly(Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester[3] were added incremental amts of up to 7000 μg of the polypeptide 1. To each tube was added the equiv point amt of the antigen (30 μg) and the tubes were then incubated at 37° for 1 hr. After standing at 4° for 48 hr, the ppts were collected, washed twice with H_2O, and collected by centrifugation. The total amt of protein pptd was estimated by N analysis by a micro-Kjaldahl method. It was found that the precipitin reaction between poly(Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester and its antisera was 50% inhibited by the addn of 750 μg of poly(Ala-Glu-Ala-Gly)-Gly-*l*-^{14}C Et ester **1.**

Acknowledgments. This work was supported by a grant from the National Science Foundation.

References

(1) B. J. Johnson and E. G. Trask, *J. Chem. Soc. C*, 2644 (1969).
(2) B. J. Johnson, *J. Pharm. Sci.*, 59, 1859 (1970).
(3) B. J. Johnson and E. G. Trask, *ibid.*, 59, 724 (1970).
(4) B. J. Johnson, *J. Med. Chem.*, 14, 488 (1971).
(5) B. J. Johnson and E. G. Trask, *ibid.*, 14, 251 (1971).
(6) B. J. Johnson and D. S. Rea, *Can. J. Chem.*, 48, 2509 (1970).
(7) B. J. Johnson and E. G. Trask, *J. Chem. Soc. C*, 2247 (1970).
(8) B. J. Johnson, *ibid. C*, 1412 (1969).
(9) P. Andrews, *Biochem. J.*, 91, 222 (1964).
(10) B. J. Johnson and N. Tsang, unpublished results.
(11) B. J. Johnson, *J. Pharm. Sci.*, 60, 332 (1971).

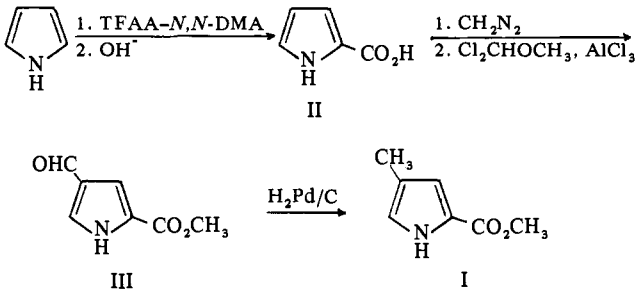
Synthesis of the Trail Marker of the Texas Leaf-Cutting Ant, *Atta texana* (Buckley)

Philip E. Sonnet

Entomology Research Division, Agricultural Research Service, Department of Agriculture, Beltsville, Maryland 20705.
Received June 24, 1971

The structure of the trail marker of the Texas leaf-cutting ant, *Atta texana* (Buckley), was recently reported by Silverstein, *et al.*, to be methyl 4-methylpyrrole-2-carboxylate, I[1] (Chart I). Although I has been previously reported,[2] the following synthesis gives a better overall yield (>60%) from available starting materials and should readily provide the quantities necessary in order to evaluate the potential usefulness to man of this type of pheromone.

Chart I



Although a number of methods are available for the preparation of pyrrole-2-carboxylic acid derivatives, for example, Ag_2O oxidation of the aldehyde[3] which is itself readily available from pyrrole,[4] a more convenient preparation is *via* the 2-CF_3CO derivative (prepared previously in 66% yield from pyrrole and $(CF_3CO)_2O$).[5] When this reaction was conducted with $PhNMe_2$ as an acid scavenger and the crude product was hydrolyzed, pyrrole-2-carboxylic acid was obtained in 85% yield from pyrrole. A similar preparation with $(Cl_3CCO)_2O$ (no added amine) has been described and gives a somewhat lower yield.[6] The acid was esterified nearly quantitatively with CH_2N_2. Alternatively it could be converted to the acid halide with oxalyl chloride and then esterified although the yield of ester was lowered thereby to 65-70%.

The 2-carbomethoxy group only deactivates the ring mildly. Villsmeier–Haack formylation and the Gatterman reaction gave predominantly the 5-formylated ester.[7,8] However, formylation of this ester with Cl_2CHOCH_3 and $AlCl_3$ has been reported parenthetically to give the desired 4-formyl derivative, III.[9] We found, in fact, that a 90% yield was obtained essentially uncontaminated by the 5 isomer (see Experimental Section).

Diborane reduction of III was complicated by self-condensation although such reductions to methyl are often successful.[10,11] Catalytic hydrogenolysis provided I in 83% yield. Laboratory tests with I on *A. texana* show that it has the activity of the natural product.

Experimental Section†

2-Pyrrolecarboxylic Acid (II) and Methyl Ester. A soln of 15.0 ml of pyrrole and 27.3 ml of $PhNMe_2$ in 150 ml of anhyd Et_2O was added dropwise to a stirred, cooled solution of 30.0 ml of $(CF_3CO)_2O$ in 375 ml of Et_2O, maintaining the temp at ≥0°. The mixt was allowed to attain ambient temp overnight, and was then washed several times with H_2O, dried ($MgSO_4$), and concd on the steam bath. Distn of the residue gave 89% of 2-trifluoroacetylpyrrole, bp 102–105° (35 mm). Glpc data (10% SE-30 on base-washed Chromosorb P at 130°) indicated >97% purity. The crude acylation product was converted to the acid, II, by placing it in a soln of 40 g of NaOH in 400 ml of EtOH–H_2O (1:1) and refluxing this soln for 3 hr. The mixt was concd to 0.5 vol, acidified with cold dil HCl, and extd with Et_2O. The ext was dried ($MgSO_4$) and concd, yielding 21.3 g (89%) of II. Esterification with CH_2N_2 in Et_2O in the usual manner provided the Me ester (96%), mp 71.5–73° (heptane), lit.[3] 72–73°.

Alternatively, the acid (22.6 g) could be stirred in a soln of 20 ml of oxalyl chloride in 300 ml of anhyd Et_2O overnight. The reaction mixt was stripped of solvent and excess oxalyl chloride (<30°). A soln of 30 ml of $PhNMe_2$ in 200 ml of MeOH was added, and the warm mixt was allowed to stand for 3 hr. It was diluted with H_2O and extd with dil HCl, H_2O, and then aq $NaHCO_3$. The soln was dried ($MgSO_4$) and concd to give 17.6 g (69%) of the crude ester.

Methyl 4-Formyl-2-pyrrolecarboxylate (III). A soln of 10.0 g of II Me ester and 25.7 g of $AlCl_3$ in 300 ml of $(ClCH_2)_2$ (EDC)–$MeNO_2$ (1:1) was chilled to −20°. A soln of 11.2 g of Cl_2CHOCH_3 in 20 ml of EDC was added fairly rapidly and the mixt was then stored at −20° overnight. It was poured over crushed ice, the layers were sepd, and the aq phase was extd with Et_2O. The combined exts were washed, dried ($MgSO_4$), and concd, yielding 10.5 g (86%) of III. Recrystn (heptane) gave mp 123.5–125°, lit.[12] 121–122°; nmr ($CDCl_3$–DMSO-d_6, trace of piperidine) 9.90 (s CHO), 7.63 (d, H 3), 7.27 (d, H 5), 3.87 (s, CH_3), J_{35} = 1.6 Hz—compares well with reported Et ester.[7] Vpc (10% SE-30 on base-washed Chromosorb P at 180°) indicated 1% contamination of crude III by the 5-formyl isomer when the reaction was conducted at 0° and essentially pure III for the reaction at −20°. Comparison was made with the known mixture derived from Villameier–Haack formylation of the ester.

Methyl 4-Methyl-2-pyrrolecarboxylate (I). Compd III (10.5 g) was dissolved in 75 ml of AcOH and treated briefly with 1 g of 10% Pd/C. The mixt was filtered and dild with 75 ml of AcOH. AcONa (4 g) and 2 g of 10% Pd/C were added and the mixture was shaken under 3.16 kg/cm² initial pressure of H_2 overnight. The mixt was

†Nmr spectra were obtained with a Varian HA-100 spectrometer (data given are in ppm relative to TMS) and ir spectra were recorded as nujol mulls or CCl_4 solutions with a Perkin-Elmer Model 521 infrared specrophotometer. Glpc data were obtained with an Aerograph Model A-700 instrument. The mention of a proprietary product in this paper does not constitute an endorsement of this product by the U. S. Department of Agriculture.