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.

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

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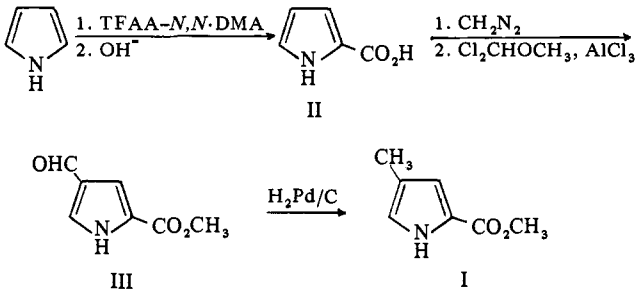
The structure of the trail marker of the Texas leaf-cutting ant, *Atta texana* (Buckley), was recently reported by Silverstein, *et al.*, to be methyl 4-methylpyrrole-2-carboxylate, I[1] (Chart I). Although I has been previously reported,[2] the following synthesis gives a better overall yield (>60%) 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.

filtered, made alk with cold aq NaOH, and extd thoroughly with Et_2O. The crude product was recrystd from hexane, giving 7.9 g (83%) of I, mp 72.5–73.5° (hexane), lit.[2] 73–74°, nmr (CCl_4) 6.62, 6.57 (H 3, H 5), 3.76 (s, CO_2CH_3), 2.04 (s, CH_3).

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Fibrin-Stabilizing Factor Inhibitors. 4. Action of Some Synthetic Fibrinoligase Inhibitors on Human Platelet Aggregation[1]

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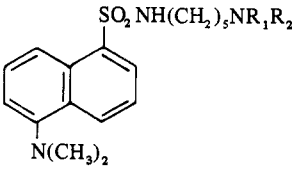
It has been shown that fibrinogen plays a role in the aggregation of platelets.[2] Therefore, we thought it would be of interest to examine the possible effect on platelet aggregation of compds which are known to inhibit specifically the enzymatic cross-linking of fibrin. In plasma, the cross-linking of fibrin is the last step in normal blood coagulation, and the reaction is catalyzed by fibrinoligase, a transamidase, which arises from the fibrin-stabilizing factor zymogen (factor XIII) through activation by thrombin.[3] The presence of a similar,[4] though not identical,[5] transamidase in platelets is well known and the requirement for thrombin has also been shown.[6]

Monodansylcadaverine [*N*-(5-aminopentyl)-5-dimethylamino-1-naphthalenesulfonamide (**1**)] is one of the most effective inhibitors of fibrinoligase.[1,7] We found that this compd also inhibited the second phase of ADP-induced aggregation of human platelets (Figure 1), whereas the initial phase was only slightly or not at all affected.

It was then pertinent to ascertain whether the effect of monodansylcadaverine on the platelet aggregation was due to its inhibitory effect on fibrin cross-linking, *i.e.*, whether it was dependent on its functional primary amino group. While several primary amines (*e.g.*, glycine methyl ester) inhibit the fibrinoligase-catalyzed reaction, the corresponding *N*-alkyl derivs (*e.g.*, sarcosine methyl ester) are ineffective.[7a] We have also shown[1] that the similarly alkylated dansyl compds **2** and **3** have no inhibitory action on fibrinoligase.



1, $R_1 = R_2 = H$
2, $R_1 = H$; $R_2 = CH_3$
3, $R_1 = R_2 = C_2H_5$

Aggregation experiments were performed on a number of plasma samples as described in the Experimental Section. Figure 1 gives a typical example of the recordings. All 3 compds showed an inhibitory effect on the second phase of the ADP-induced platelet aggregation. There is some suggestion that monodansylcadaverine (**1**) may be more effective than the two analogs (**2**, **3**).

Inhibition by monodansylcadaverine was even more pronounced when norepinephrine was used to initiate platelet aggregation (Figure 2). In this case, already the first phase of aggregation seemed to be inhibited.

It can thus be concluded that monodansylcadaverine inhibits both the ADP- and norepinephrine-induced aggregation of human platelets. Since the compds with alkylated aliphatic amino groups (**2**, **3**) act similarly, the primary amino group which is essential for inhibiting fibrin cross-linking, does not seem to be involved in the inhibition of platelet aggregation. Similar alkylated amines have recently been studied by Lacefield, *et al.*,[8] and shown to inhibit platelet aggregation.

Experimental Section

Biological Methods and Materials. The method of Born was followed for measuring platelet aggregation,[9] using an EEL titrator equipped with a magnetic stirrer and thermostated at 37° (±0.2°). The titrator was coupled to a Labograph E 478, Methrohm AG recorder.

All glassware used in contact with blood or plasma samples was siliconized. Fresh human blood was drawn into 3.8% sodium citrate

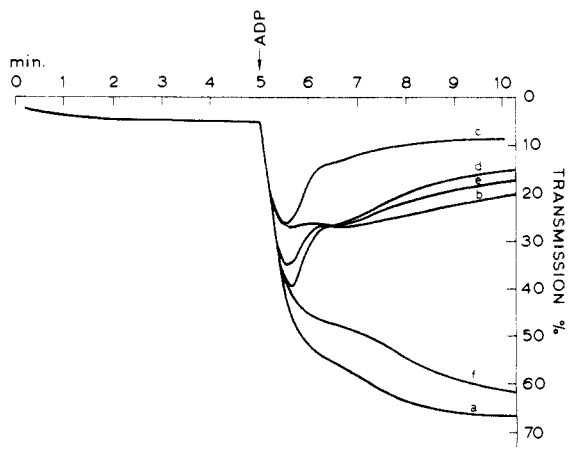


Figure 1. Effects of dansylcadaverine (**1**) and **2** and 3 on ADP-induced platelet aggregation. Inhibitor, $3.1 \times 10^{-4} M$; ADP, $3.1 \times 10^{-6} M$. Curve a and f: no inhibitor, a recorded at the start and f at the end of the expt. Curve: b, adenosine ($3.1 \times 10^{-6} M$); c, dansylcadaverine (**1**); d, compd **2**; e, compd 3.