Synthetic Analogs of the Trail Pheromone of the Leaf-Cutting Ant, *Atta texana* (Buckley)

Philip E. Sonnet* and John C. Moser[1]

The preparation and bioassay of a number of analogs of the trail-following pheromone of the town ant, *Atta texana* (Buckley), are described. Although several compounds were quite active, isomers of the pheromone were not. The results are discussed with a view to the probable conformations of these molecules.

Investigations of the town ant, *Atta texana* (Buckley), revealed the structures of its alarm pheromones (Moser *et al.*, 1968) and one of its trail-following pheromones (Tumlinson *et al.*, 1971; Wood *et al.*, 1970). This ant is a pest of pine plantations and farm crops (Moser, 1962); hence chemicals which control its social behavior are of great concern. We have prepared a number of analogs of the trail-following pheromone, methyl 4-methylpyrrole-2-carboxylate (V), and have examined their biological activities.

Furthermore, considerable interest exists in the mechanisms of insect olfaction (Schneider, 1971). Recent exciting work (Amoore *et al.*, 1969; Blum *et al.*, 1971; Tai *et al.*, 1971) has stressed the importance of conformational analysis in divining structure–activity relationships. The fairly rigid structure of V and its analogs greatly reduces the number of conformations with which one must contend. This should make arguments based on conformation somewhat more binding than those which have been applied recently to alarm (Amoore *et al.*, 1969), sex (Blum *et al.*, 1971), and trail-following pheromones (Tai *et al.*, 1971).

EXPERIMENTAL SECTION

The syntheses are outlined in the Synthetic Scheme. Infrared spectra were obtained with a Perkin-Elmer Model 137 spectrophotometer. Nuclear magnetic resonance spectra were measured with a Varian T-60 instrument and gas chromatographic analyses were performed with an Aerograph Model A-700 instrument employing various columns. Chemical analyses were obtained for all new compounds and melting point comparisons with literature values were made for compounds previously reported.

We first prepared all of the isomers of the trail-marking compound V. The source of I and II (Figure 1) was 2-methylpyrrole (Treibs and Ott, 1958). Similarly, III and IV were obtained from 3-methylpyrrole (Lancaster and Vander Werf, 1958). Prior art with 3-methylpyrrole indicated that predominant attack by electrophiles at position 2 was expected (Badger *et al.*, 1964; Rapoport and Bordner, 1964; Meinwald and Meinwald, 1966). Thus, phosgenation provided a mixture of IV and V (~9:1) as determined by gas chromatography and nuclear magnetic resonance spectroscopy. The Grignard reagent derived from 3-methylpyrrole provided a greater proportion of the 5-substituted product when it was acetylated. Thus, a mixture of VI and VII was obtained in the ratio of 6:4. Compound VI was obtained pure by fractional crystallization, whereas compound VII was obtained only as a mixture with VI.

Compound IX was prepared from methyl pyrrole-3-carboxylate (VIII) (Rapoport and Willson, 1961). The latter was also formylated to give X, which was hydrogenated to XI. Compound XII was obtained from ethyl 2-methylpyrrole-3-carboxylate (Treibs and Ott, 1958) by saponification and methylation of the resulting acid.

The synthesis of V has been described (Sonnet, 1972a); its preparation served to produce XIII and XIV. *N*-Methylation of V gave XV. The analogous procedure with methylpyrrole-2-carboxylate gave XVI. Acetylation of XIII gave XVII. The acetylation employed (acetyl chloride, aluminum chloride, 1:1 nitromethane–ethylene dichloride, −20°) provided a material containing no more than 1% of the 5-acetyl compound (Anderson and Huang, 1967). Diborane reduction of XVII gave XVIII.

The acid XIX served as a source of esters XX–XXII, the thiol ester XXIII, and amides XXV and XXVI. The aldehyde XXIV was prepared from V *via* the hydrazide.

Pyrrole-2-carboxaldehyde, XXVII (Silverstein *et al.*, 1956), was converted to a ternary iminium salt (Sonnet, 1971) which served as a source for bromoaldehyde (XXVIII), chloroaldehyde (XXX), and dialdehyde (XXXII) (Sonnet, 1972b). Each of these compounds was subsequently oxidized with silver oxide to the corresponding acid (diacid) and esterified to the methyl ester (diester).

Compounds XXXIV (Rapoport and Bordner, 1964) and XXV (Khan *et al.*, 1966) were prepared essentially as described in the literature.

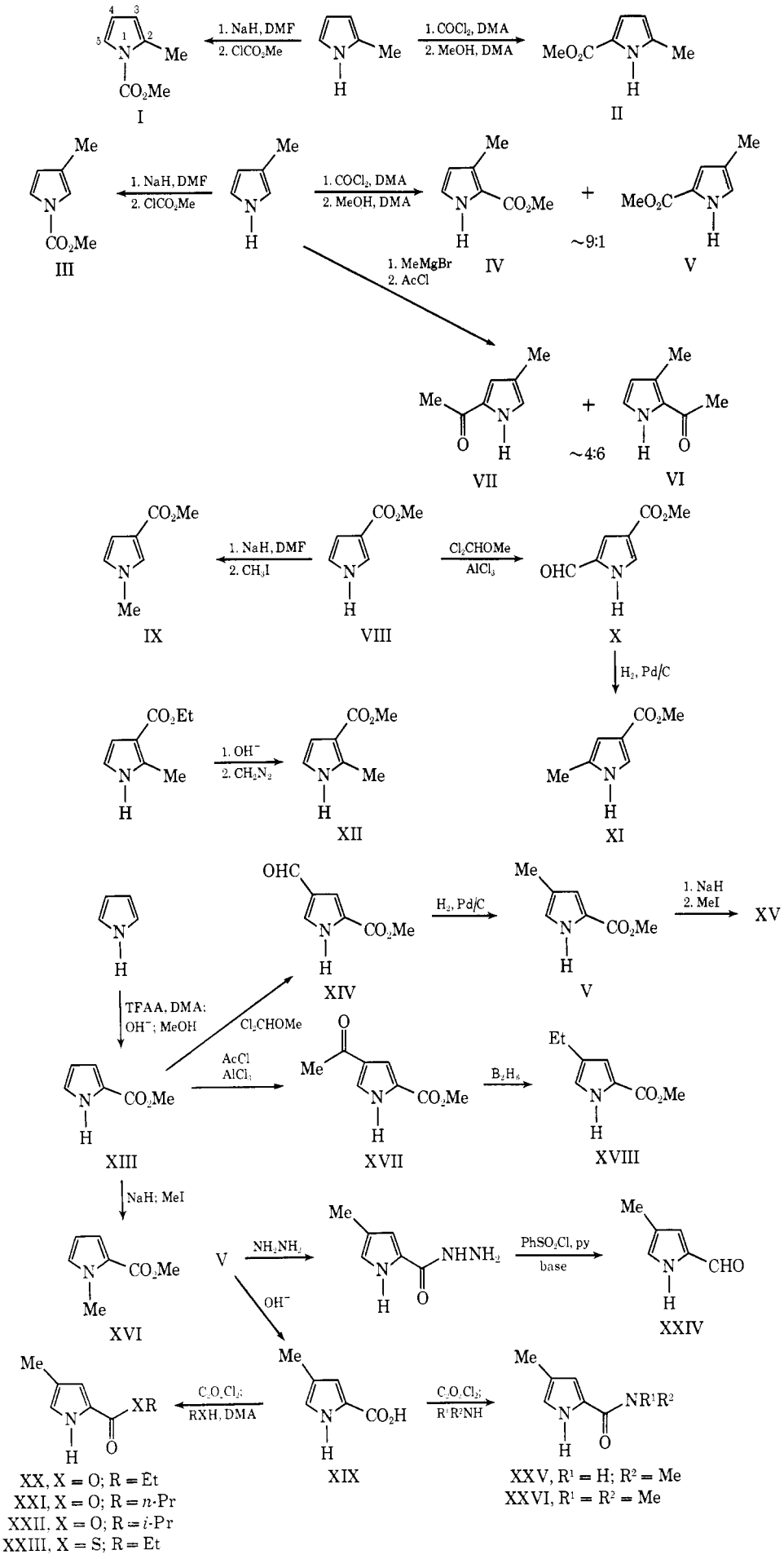
Testing Method. Circles, 50 cm in circumference, were described on slick cardboard sheets with 10-μl chloroform solutions of synthetics serially diluted to 40 mg/μl, 0.4 ng/μl, and 0.004 ng/μl. These concentrations embrace the range of activity for compound V. Repulsion was observed at the highest of these concentrations. Fifteen minor workers from a laboratory ant colony were then released into the center of the circle. The potency of the synthetic chemical was evaluated by counting the first ten ants that followed the trail or crossed the circle edge.

RESULTS AND DISCUSSION

The data are summarized in Table I. None of the isomers of V (I–IV, IX, XI, XII, XVI, and XXXIV) showed any significant activity. Positional isomers, such as I and IV, in which the methyl and carbomethoxy groups are on adjacent ring positions, have a shape obviously different from V. It was not surprising that they were inactive. Isomers bearing these

U. S. Department of Agriculture, Agricultural Research Service, Entomology Research Division, Beltsville, Maryland 20705.

[1] U. S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, Pineville, Louisiana 71360.


1. NaH, DMF
2. ClCO2Me
1. COCl2, DMA
2. MeOH, DMA
I
II
III
IV
V
~9:1
1. MeMgBr
2. AcCl
VII
VI
~4:6
1. NaH, DMF
2. CH3I
Cl2CHOMe
AlCl3
IX
VIII
X
H2, Pd/C
1. OH−
2. CH2N2
XII
XI
TFAA, DMA;
OH−; MeOH
AcCl
AlCl3
B2H6
XIII
XVII
XVIII
NaH; MeI
XVI
NH2NH2
PhSO2Cl, py
base
XXIV
C2O2Cl2
RXH, DMA
XIX
R1R2NH
XX, X = O; R = Et
XXI, X = O; R = n-Pr
XXII, X = O; R = i-Pr
XXIII, X = S; R = Et
XXV, R1 = H; R2 = Me
XXVI, R1 = R2 = Me

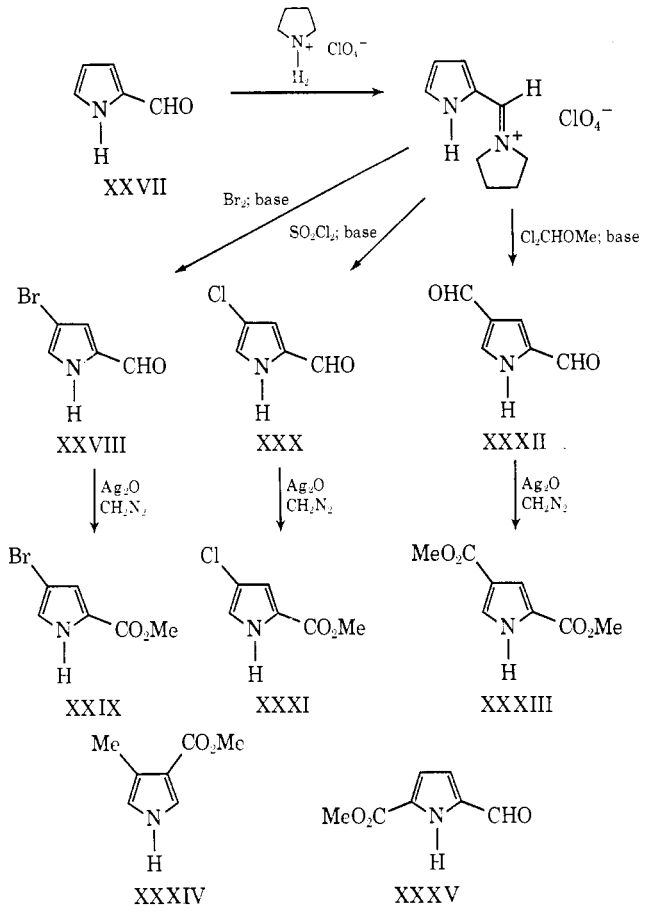


Figure 1. Synthesis of analogs of trail pheromone (above and left)

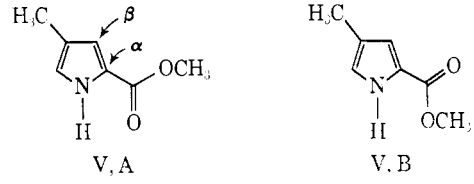


Figure 2. The conformers of V

substituents in a 1,3 relationship, such as II and XI, were also inactive, however. Such compounds appear to approximate the steric requirements of V, *i.e.*, the pyrrole ring is treated here as a nearly regular pentagon (Jones, 1970); and at this point a discussion of the conformational preferences of these pyrroles is in order.

Compound V is essentially a planar molecule. The atoms of the ring and the bonds to groups attached to the ring lie in a plane. The carbomethoxy group is in electronic conjugation with the ring. Presumably the carbonyl carbon and both oxygen atoms are coplanar with the ring.

The structure of V may therefore be drawn as VA or VB (Figure 2). An infrared study of methyl 2-pyrrolecarboxylate (Jones and Moritz, 1965), *i.e.*, V minus the ring methyl group, indicated the existence of both A and B. Studies of the conformations of pyrrole-2-carboxaldehyde (Gronowitz *et al.*, 1961; Jones and Wright, 1968) indicated a preference for the equivalent structure for A (H instead of OCH_3) and a more recent examination of 1-methyl-4-bromo-2-pyrrolecarboxaldehyde (Roques *et al.*, 1971) showed that although A was preferred at room temperature by about 9:1 over B, these conformations were rapidly interconverting.

Table I. Activities of the Pyrroles[a]

Compound		Activity, ng/μl 40	0.4	0.004
Isomers				
V		R	+++	+
I		−	−	−
II		−	−	−
III[b]		R	−	−
IV[c]		R	++	−
IX		−	−	−
XI[d]		R	−	−
XII		−	−	−
XVI		−	−	−
XXXIV		R	−	−
Changes in the 4 position				
XIII	(H)	−	−	−
XIV	(CHO)	++	+	−
XVII	($COCH_3$)	−	−	−
XVIII	(C_2H_5)	−	++	−
XXIX	(Br)	++	+++	−
XXXI	(Cl)	R	+++	++
XXXIII	(CO_2CH_3)	+	−	−
Changes in the 2 position				
VII[e]	($COCH_3$)	+	+	−
XIX	(CO_2H)	−	−	−
XX	($CO_2C_2H_5$)	−	−	−
XXI	(CO_2-*r*-C_3H_7)	−	−	−
XXII	(CO_2-*i*-C_3H_7)	−	−	−
XXIII	($COSC_2H_5$)	−	−	−
XXIV	(CHO)	++	−	−
XXV	($CONHCH_3$)	−	−	−
XXVI	($CON[CH_3]_2$)	−	−	−
Other 2-aldehydes				
X	(4-CO_2CH_3)	−	−	−
XXVII	(4-H)	−	−	−
XXVIII	(4-Br)	+++	−	−
XXX	(4-Cl)	++	−	−
XXXII	(4-CHO)	+	−	−
Miscellaneous				
VI	(2-$COCH_3$-3-CH_3)	−	−	−
VIII	(3-CO_2CH_3)	R	−	−
XV	(NCH_3V)	−	−	−
XXXV[f]	(2-CO_2CH_3-5-CHO)	−	−	−

[a] The scoring was: −, 0–2 ants following the trail; +, 3–4 ants; ++, 5–7 ants; +++, 8–10 ants. The bioassay was reasonably reproducible and repetitions rarely gave a change in response amounting to more than a single (+) difference. R indicates repulsion. [b] Repulsion was not reproduced in a subsequent test. No positive response observed. [c] This compound could not be freed of V, which was a contaminant in its preparation. On the basis of the other screening results, the activity observed is undoubtedly due to V. [d] Repulsion was reproducible and a (+) response was observed at 4.0 ng/μl. [e] Tested as a 1:1 mixture with VI. The activity is due to VII and indicates lower activity for the acetyl group than the carbomethoxy group. [f] Obtained as a mixture with XIV. Repeated column chromatography gave a sample of sufficient purity (>99.5%) to indicate inactivity of XXXV.

A receptor site sensitive to physical dimensions alone might fail to distinguish the pyrrole nitrogen from the ring carbon atoms. An examination of the two analogous conformations of II shows that each one is identical in size and shape to one of the conformers of V (Figure 3). Clearly the same statement can be made for each of the isomers in which the methyl and carbomethoxy groups are 1,3 on the ring. Since each of these isomers can achieve the required shape, either A or B, their inactivity is evidence for another variable. Evidently the receptor site can distinguish the pyrrolic nitrogen from the ring carbons and this suggests immediately that electronic interactions (complexation or bonding) may occur in these sites. The requirement for the carbomethoxy group adjacent to the pyrrolic nitrogen with a methyl group in a 1,3 relationship to either of these makes V distinct from its isomers.

The *N*-methylated V (XV) was inactive. A number of other trisubstituted pyrroles were also screened and were

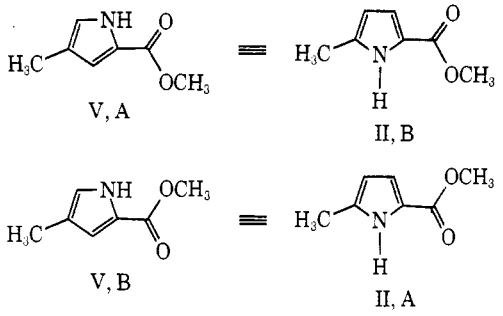


Figure 3. Comparison of conformations of II and V

found inactive. Replacement of the carbomethoxy group with the carboxaldehyde (XXIV) or acetyl group (VII) retained activity, but the ethyl ester was inactive. Enlargement of the group at the 2 position cannot be tolerated, but a smaller group with similar electronic properties can.

Loss of the methyl group deactivated the molecule (note that reduction in size is adverse at this position and involves a chemically unreactive group). However, other units (Cl, Br, C_2H_5, even the polar CHO and CO_2CH_3) did not prevent the molecule from stimulating the receptor site into evoking a trail-following response. Therefore, there is less restriction on the size and polarity of the group at the 4 position than at the 1 or 2 positions. Perhaps this unit serves primarily to orient the molecule within the receptor (a coarse adjustment).

Compound XIX with a free carboxyl group at the 2 position was not active. The amides XXV and XXVI were also inactive. The amides have not yet been examined for conformational preference, but the nmr spectrum of XXV indicates hindered rotation about the –C(=O)N bond (2-*N*-methyl absorptions). The conjugation of amide nitrogen with the carbonyl group implied by this observation indicates little conjugation of the ring with the carbonyl group. Hence XXV should be capable of attaining either the A or B conformation, *i.e.*, rotation about the CC(=O)N bond may be even less restricted than for V. Hence the inability of XXV to achieve a required shape is probably not responsible for its inactivity.

CONCLUSIONS

A current concept of insect olfaction is the fitting of a preferred conformer into a rigid three-dimensional receptor site. However, if conformational equilibration is rapid by comparison with the physical or chemical reactions of the candidate with the receptor site, no evaluation of the shape of the site can be made based on structure of a predominant conformer. In the present study, conformational analyses suggest that the ability to attain a specific size and shape is insufficient to cause pheromonal response. The uniqueness of V as compared to its isomers and the general requirement of 2,4 substitution on the pyrrole ring suggest that bonding occurs in the receptor site to actuate the olfactory response.

LITERATURE CITED

Amoore, J. E., Palmieri, G., Wanke, E., Blum, M. S., *Science* **165,** 1266 (1969).
Anderson, H. J., Huang, C. W., *Science* **45,** 897 (1967).
Badger, G. M., Harris, R. L. N., Jones, R. A., *Aust. J. Chem.* **17,** 1022 (1964).
Blum, M. S., Boch, R., Doolittle, R. E., Tribble, M. T., Traynham, J. G., *J. Insect Physiol.* **17,** 349 (1971).
Gronowitz, S., Hörnfeldt, A. B., Bestblom, G., Hoffman, R. A., *Ark. Kemi* **18,** 133 (1961).
Jones, R. A., Moritz, A. G., *Spectrochim. Acta* **21,** 295 (1965).
Jones, R. A., Wright, P. H., *Tetrahedron Lett.* 5495 (1968).
Jones, R. A., *Advan. Heterocycl. Chem.* **11,** 383 (1970).
Khan, M. K. A., Morgan, K. J., Morrey, D. P., *Tetrahedron* **22,** 2095 (1966).
Lancaster, R. E., Jr., Vander Werf, C. A., *J. Org. Chem.* **23,** 1208 (1958).
Meinwald, J., Meinwald, Y. C., *J. Amer. Chem. Soc.* **88,** 1305 (1966).
Moser, J. C., *Forests People* **12,** 12 (1962).
Moser, J. C., Brownlee, R. C., Silverstein, R. M., *J. Insect Physiol.* **14,** 529 (1968).
Rapoport, H., Willson, C. D., *J. Org. Chem.* **26,** 1102 (1961).
Rapoport, H., Bordner, J., *J. Org. Chem.* **29,** 2727 (1964).
Roques, B., Jaurequiberry, C., Fournié-Zaluski, M. C., Combrisson, S., *Tetrahedron Lett.* 2693 (1971).
Schneider, D., *Naturwissenschaften* **58,** 194 (1971).
Silverstein, R. M., Ryskiewicz, E. E., Willard, C., *Org. Syn.* **36,** 74 (1956).
Sonnet, P. E., *J. Org. Chem.* **36,** 1005 (1971).
Sonnet, P. E., *J. Med. Chem.* **15,** 97 (1972a).
Sonnet, P. E., *J. Org. Chem.* **37,** 925 (1972b).
Tai, A., Matsumura, F., Coppel, H. C., *J. Insect Physiol.* **17,** 181 (1971).
Treibs, A., Ott, W., *Justus Liebigs Ann. Chem.* **615,** 137 (1958).
Tumlinson, J. H., Moser, J. C., Silverstein, R. M., Brownlee, R. G., Ruth, J. M., *Nature (London)* **234,** 348 (1971).
Wood, D. L., Silverstein, R. M., Nakajima, M., Ed., "Control of Insect Behavior by Natural Products," Academic Press, New York, N. Y., 1970, pp 161–178.

Received for review June 6, 1972. Accepted August 3, 1972.