areal return current under it on the surface of the earth. This requirement is satisfied if a horizontal image current 6 km below the surface is chosen. Thus, for a measured B of 15 gammas, I equals 225 amperes. This value represents the minimum current needed to produce the measured magnetic deflection. In the model chosen, B exhibits essentially $1/R$ dependence. In a ring-current model, for example, such as one might postulate to be rotating with the funnel, the magnetic field would vary at least as $1/R^2$, requiring the existence of much higher currents.

Whatever the model chosen to explain the magnetic effects, the existence of high currents of the order of hundreds of amperes is implied, and the energy involved is staggering. For example, if one extrapolates from the ordinary thunderstorm and chooses a value of 10^8 volts for the potential difference between cloud and ground, the electrical energy is calculated as being dissipated at a rate of 2.25×10^8 joules/second. Over a period of 5 minutes, approximately 7×10^{12} joules of energy will have been expended.

An average current of 225 amp must, at first sight, appear incredible. But long continuing currents in lightning from ordinary thunderstorms have been measured at greater than 400 amp for durations exceeding 1 second. An average thunderstorm, however, involves an equivalent steady current of about 1 amp. From the results based upon the assumed model, one can infer that a tornado is equivalent to several hundred thunderstorm cells active simultaneously.

A current of 225 amp flowing for 10 minutes involves a total charge of 135,000 coulombs, or about one-third of the total charge on the surface of the earth. The release of charge in this amount should be detectable anywhere on earth, provided that local effects in the atmospheric electric field are small. Electric field or air-earth current measurements several hundred miles from a tornado should show this effect if the currents inferred from the magnetic field measurements are as large as calculated. In support of this inference, Falconer and Schaefer (*5*) detected strong negative electric fields in Schenectady, N.Y. during the period of the Worcester tornado on 9 June 1953, when the sky was clear overhead and the tornado was 150 km distant.

Although this measurement represents only one observation, there may exist other records such as this one which have not been reported. My primary purpose in writing this note is to bring to the attention of scientists, especially those who live in areas of frequent tornado occurrence that reliable magnetic measurements made in the vicinity of tornadoes would be most helpful in establishing whether or not electrical effects are important in the energy budget of the tornado. It would be highly desirable to have an extended array of three component magnetometers. Data from such an array should make possible the construction of a reasonably consistent model of the distribution and magnitude of the large currents which seem to accompany a tornado.

MARX BROOK
New Mexico Institute of Mining and Technology, Socorro

References and Notes

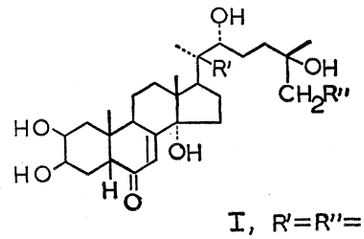
1. B. Vonnegut, *J. Geophys. Res.* **65**, 203 (1960).
2. ——— and J. R. Weyer, *Science* **153**, 1213 (1966).
3. Donald P. Williams and M. Brook, *J. Geophys. Res.* **68**, 3243 (1963).
4. M. Brook and B. Vonnegut, *ibid.* **65**, 1303 (1960).
5. R. E. Falconer and V. J. Schaefer, *Bull. Amer. Meteorol. Soc.* **35**, 437 (1954).
6. I thank Mr. Geoffrey Boucher for allowing me to use his records.

19 June 1967

Insect Hormones: Alpha Ecdysone and 20-Hydroxyecdysone in Bracken Fern

Abstract. *The two major molting hormones of insects, alpha ecdysone and 20-hydroxyecdysone, were isolated in crystalline form from dry pinnae of the bracken fern,* Pteridium aquilinum *(L.) Kuhn. Three unidentified substances with molting hormone activity were also detected. Bracken is the first plant found to contain both of the major insect ecdysones, and it is the first known plant source of alpha ecdysone.*

Three structurally related hormones, termed ecdysones, that regulate molting and metamorphosis in insects have been isolated and identified. Alpha ecdysone (*1*), the first molting hormone isolated, was shown by x-ray diffraction studies (*2*) and by syntheses (*3*) to be a compound of structure I. The 20-hydroxyecdysone of structure II has been isolated from insects (*4, 5*) and from crustacea (*6*), and a third hormone 20,26-dihydroxyecdysone of structure III was isolated (*7*) from the tobacco hornworm pupa, *Manduca sexta* (Johannson). The configurations at C-20 and C-22 in II and III remain to be established.



I, R'=R''=H
II, R'=OH; R''=H
III, R'=R''=OH

Reports that two Coniferophytes also yield similar or related steroids (*8, 9*) with molting hormone activity in extremely large quantities prompted us to examine certain more primitive plants. Ferns, which are widely distributed and relatively immune to insect attack (*10*), were considered ideal subjects for these studies.

During the winter in the vicinity of the laboratory, we observed stands of withered, but intact, bracken *Pteridium aquilinum* (L.) Kuhn. Crude extracts from the dry pinnae proved positive in the housefly assay (*11*), and the titer was greater than that found for 7-day-old tobacco hornworm pupae (*5*), the best insect source of the ecdysones yet reported.

A sufficient quantity of the dry bracken was collected in mid-January; the pinnae were separated from the plant and pulverized to a powder. The dry powder (4.0 kg) was blended once with 75 percent methanol (10 ml per gram) and then twice again with 5 ml per gram. The techniques used to isolate and purify the extracted, biologically active components were similar to those used to isolate the ecdysones from insect sources (*5*). The adsorption columns (*12*) were scaled to accommodate larger quantities of extractives, and fractions were monitored by ultraviolet spectroscopy and by bioassay.

The concentrate, after adsorption chromatography, was subjected to 37 transfers in a countercurrent distribution system consisting of cyclohexane,

butonal, and water (5 : 5 : 10) (*5*); and in this solvent system two major peaks (tubes 8 to 17, and 22 to 29) were detected by the housefly assay. Preparative thin-layer chromatography (TLC) (*7*) of the pooled fractions representing the faster-moving, less-polar peak in countercurrent distribution gave a major zone with an R_F similar to α-ecdysone and also three additional minor zones with activity. The zone corresponding to α-ecdysone yielded 1.8 mg of crystalline material. Preparative TLC of the more polar material from countercurrent distribution gave a zone with an R_F value similar to that of 20-hydroxyecdysone. This zone, which contained the only active principle on the plate, yielded 4.0 mg of crystalline material. Mass spectroscopy of the crystalline apolar and polar compounds from the fern indicated molecular weights of 464 and 480 and gave fragmentation patterns identical to those of α-ecdysone (I) and 20-hydroxyecdysone (II), respectively. Other physical properties and spectral data (Table 1) confirm that the two compounds isolated from bracken are α-ecdysone and 20-hydroxyecdysone. Bioassay of the crystalline hormones from bracken showed these to be equal in activity to the authentic α-ecdysone and 20-hydroxyecdysone isolated from insects (Table 2).

Bracken, then, is the first plant found to contain both major insect ecdysones and is the first known plant source of α-ecdysone. The presence of α-ecdysone and 20-hydroxyecdysone in bracken suggests similar metabolic pathways for steroids in the insect and in this fern. Identification of the three biologically active unknowns could provide information on the biosynthesis metabolism of the ecdysones in insects.

The 20-hydroxyecdysone has been reported from two other plant sources, a gymnosperm, *Podocarpus elata* R. Br. ex Mirb. (*9*), and an angiosperm, *Achyranthes radix* (*13*), and its presence in bracken fern now places this steroid hormone of insects and crustacea in a representative plant from each of the three classes of the Pteropsida. The ecdysones which were previously thought to be specific in insects are now being detected in a variety of living systems.

We found molting hormone activity in crude extracts of pinnae from the four other species of ferns we examined; the sensitive fern *Onoclea sensibilis* L., Christmas fern *Polystichum acrostichoides* (Michx.) Schott, common polypody *Polypodium virginianum* L., and cinnamon fern *Osmunda cinnamomea* L. The titer, however, varied considerably among the different species of ferns which could reflect a qualitative, as well as a quantitative, difference in the ecdysones present.

Four biologically active substances with molting hormone activity have been isolated from the leaves of a yew, *Podocarpus nakaii* Hay (*8*). The major component, identified as 25-deoxy-20-hydroxyecdysone, has activity approaching that of the insect ecdysones (*14*). The other two substances tested, however, were 1/10 and 1/1000 as active as 25-deoxy-20-hydroxyecdysone; this indicates that these compounds are not identical to either α-ecdysone or 20-hydroxyecdysone. Our detection of three active unknown components in bracken still presents the possibility that the bracken and *Podocarpus nakaii* may have certain ecdysones in common.

The role of the ecdysones in insects has been rather extensively studied (*15*). However, we do not have information on either the significance or function of these steroids in plants. Perhaps, like the estrogens that have been reported to stimulate germination and growth (*16*), these substances may also have a specific physiological role in the developing fern, or perhaps these biologically active substances have been elaborated by the plant to interfere with the growth processes of insect predators (*9*). Such a role has also been postulated (*17*) for the juvenile hormone mimic juvabione (*18*) which is present in the Balsam fir *Abies balsamea* (L.) Miller. The presence of the major insect ecdysones in bracken and the relative immunity of ferns to insect attack strongly suggest that the study of these steroids in ferns will provide information on the role or roles that these hormonal substances may play in insect-host plant interrelationships.

Table 1. Physical properties of α-ecdysone and 20-hydroxyecdysone from bracken compared with authentic standards. λ, Wavelength of absorption; ϵ, molar extinction coefficient; TLC, thin-layer chromatography.

Ecdysones	Melting point (°C)	Methanol		Nuclear magnetic resonance,* methyl resonances (cycle/sec)				Infrared in KBr (cm^{-1})	TLC (R_F)
		λ_{max}	ϵ_{max}	18-H	19-H	21-H	26-H and 27-H		
				Bracken					
α-Ecdysone	232–235	244	11,600	44	64	72, 78	82	1658, 1652	0.34
20-Hydroxy-ecdysone	230–234	244	12,300	73	64.5	94	82	1658, 1652	0.23
				Standards					
α-Ecdysone	237–239	245	12,018	43.5	64.5	73, 78	82	1658, 1652	0.34
20-Hydroxy-ecdysone	233–235	245	12,557	73	64.5	94	82	1658, 1652	0.23

* Recorded at 60 megacyles in deuteriopyridine with tetramethylsilane as an internal standard.

Table 2. Comparison of the biological activity of α-ecdysone (α-Ecd) and 20-hydroxyecdysone (20-OH-Ecd isolated from bracken with authentic standards by the housefly assay. The percentage of response is based on the average from two groups of 15 insects.

Dose (μg)	Percentage of response			
	Bracken		Standards	
	α-Ecd	20-OH-Ecd	20-OH-α-Ecd	Ecd
0.0075	62	66	64	70
0.0050	40	52	44	57
0.0025	20	23	12	28

J. N. KAPLANIS, M. J. THOMPSON
W. E. ROBBINS, B. M. BRYCE
Insect Physiology Laboratory,
Agricultural Research Service,
U.S. Department of Agriculture,
Beltsville, Maryland 20705

References and Notes

1. A. Butenandt and P. Karlson, *Z. Naturforsch.* **9b**, 389 (1954). We retain the designation α-ecdysone for structure I to avoid confusion with the term "ecdysones" which is a generic designation for the number of related steroids and which is often used interchangeably with "molting hormones."
2. R. Huber and W. Hoppe, *Chem. Ber.* **98**, 2403 (1965).
3. U. Kerb, P. Hocks, R. Wiechert, A. Furlenmeier, A. Furst, A. Langemann, G. Waldvogel, *Tetrahedron Lett.* No. 13, 1387 (1966); I. T. Harrison, J. B. Siddall, J. H. Fried, *ibid.* No. 29, 3457 (1966).
4. P. Hocks and R. Wiechert, *ibid.* No. 26, 2989 (1966); P. Hocks, G. Schulz, P. Karlson, *Naturwissenschaften* **54**, 44 (1967; H. Hoffmeister and H. F. Grutzmacher, *Tetrahedron Lett.* No. 33, 4017 (1966).
5. J. N. Kaplanis, M. J. Thompson, R. T. Yamamoto, W. E. Robbins, S. J. Louloudes, *Steroids* **8**, 605 (1966).
6. F. Hampshire and D. H. S. Horn, *Chem. Commun.* No. 2, 37 (1966).
7. M. J. Thompson, J. N. Kaplanis, W. E. Robbins. R. T. Yamamoto, *ibid.* No. 13, 650 (1967).

8. K. Nakanishi, M. Koreeda, S. Sasaki, M. L. Chang, H. Y. Hsu, *ibid.* No. 24, 915 (1966).
9. M. N. Galbraith and D. H. S. Horn, *ibid.* No. 24, 905 (1966).
10. C. F. Soo Hoo and G. Fraenkel, *Ann. Entomol. Soc. Amer.* **57**, 788 (1964).
11. J. N. Kaplanis, L. A. Tabor, M. J. Thompson, W. E. Robbins, T. J. Shortino, *Steroids* **8**, 625 (1966).
12. Adsorbents used were Woelm neutral grade-I alumina deactivated with 20 percent water and silicic acid (Unisil, 100 to 200 mesh, Clarkson Chemical Co., Williamsport, Pa.). Chromatography on silica gel previously used in fractionating insect material (*5*) was omitted.
13 T. Takemoto, S. Ogawa, N. Nishimoto, *Zakugaku Zasshi*, **87**, 325 (1967). After submitting the manuscript, we found that 20-hydroxyecdysone has also been isolated from another fern, *Polypodium vulgare* (L.), by J. Jizba, V. Herout, F. Sorm [*Tetrahedron Lett.* No. 18, 1689 (1967)].
14. M. Kobayashi, K. Nakanishi, M. Koreeda, *Steroids* **9**, 529 (1967).
15. P. Karlson, *Naturwissenschaften* **53**, 445 (1966).
16. E. Heftmann, *Ann. Rev. Plant Physiol.* **14**, 225 (1963).
17. K. Slama and C. M. Williams, *Biol. Bull.* **130**, 235 (1966).
18. W. S. Bowers, H. M. Fales, M. J. Thompson, E. C. Uebel, *Science* **154**, 1020 (1966).
19. We thank L. Tabor, P. Sterling, and E. Jensen for technical assistance; Drs. C. Morton and D. B. Lellinger, Smithsonian Institution, for identification of ferns; and Drs. F. Schaub and D. Merry of LKB Instruments, Inc., for the mass spectra.

12 July 1967

DDT: Interaction with Nerve Membrane Conductance Changes

Abstract. *The falling phase of action potentials of lobster giant axons is prolonged by DDT; finally a plateau phase is produced like cardiac action potentials. In axons poisoned with DDT, peak transient (sodium) currents associated with step depolarizations are turned off very slowly, and steady-state (potassium) currents are markedly suppressed. These two changes would cause the prolongation of action potentials and are considered the major ionic mechanisms of DDT action.*

It has long been known that DDT prolongs the falling phase of nerve action potentials. Shanes (*1*) first observed the prolongation of negative after-potentials in external recordings from crab nerves poisoned with DDT, and this has been confirmed with cockroach nerve (*2*). Microelectrode recordings from cockroach giant axons poisoned with DDT have revealed that delayed rectification is suppressed and that the increased negative afterpotential is further augmented and prolonged, forming a plateau resembling action potentials of cardiac muscle upon removal of potassium from the bathing medium (*3*). It has been suggested that the increase in nerve membrane conductance to potassium, or the inactivation of the nerve membrane conductance to sodium, or both are inhibited, thereby increasing the negative afterpotential (*3*). To test this hypothesis, we have performed voltage-clamp experiments with the axons poisoned with DDT. Our results unequivocally point to this as the major ionic mechanism of DDT action on nerve, and it turns out that DDT may become one of the most interesting chemicals as a tool in electrophysiology.

The giant axons in the circumoesophageal connectives of the lobster *Homarus americanus* were used. Because no data were available on the effect of DDT on lobster axons, changes in action potential were first observed in the partially isolated giant axons by means of intracellular capillary microelectrodes filled with 3*M* KCl. The microelectrode experiments were done at room temperature (22°C). The sucrose-gap voltage-clamp method with the completely isolated giant axons was essentially the same as that described for squid axons (*4*), except that the chamber was modified to adapt to lobster axons. The voltage-clamp experiments were done at 7° to 10°C.

Artificial seawater containing 468 mmole of Na^+, 10 mmole of K^+, 25 mmole of Ca^{2+}, 8 mmole of Mg^{2+}, 533 mmole of Cl^-, 4 mmole of SO_4^{2-}, and 2.5 mmole of HCO_3^- per liter at *p*H 7.9 was used as the bathing medium. Purified *p,p'*-DDT was dissolved in ethanol to make up stock solution, which was in turn injected into seawater to give a suspension of 5×10^{-4} mole of DDT per liter. The concentration of ethanol was 1 percent (by volume) and in other experiments had little or no effect on the excitability of nerve.

After the lobster axon was treated with $5 \times 10^{-4}M$ DDT, its action potential was greatly augmented and prolonged, forming a plateau phase. Repetitive afterdischarge was very often superimposed on the plateau. It usually took over 20 minutes in the isolated single axons and over 1 hour in the partially isolated axons for this change of action potential to occur. The duration of the plateau depended partly on the membrane potential; when the membrane was previously hyperpolarized by an inward polarizing current passed through a second microelectrode inserted near the voltage recording microelectrode, the plateau was further prolonged, but there was

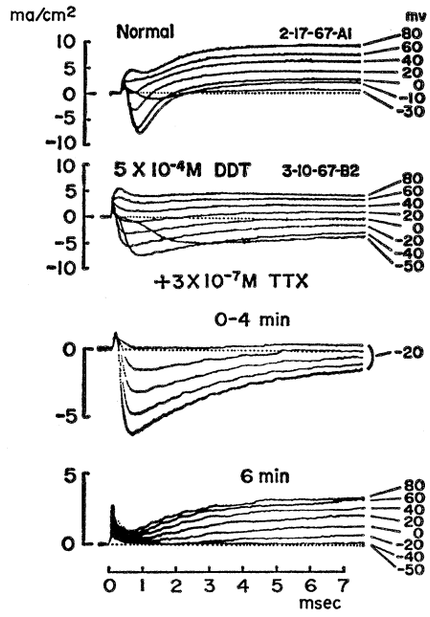


Fig. 1. Families of membrane currents associated with step depolarizations in normal axons, and those treated with DDT and with DDT and TTX. The third set of records shows changes in current during the course of TTX action. The dotted lines in each set refer to the zero base line.

a critical hyperpolarization beyond which the plateau started shortening. The absolute membrane potential at the plateau was kept almost constant by the hyperpolarization, as was the membrane potential at the peak of action potential. A change in membrane potential was also observed in seawater with DDT and free of K^+; upon removal of K from the bathing medium, the membrane was hyperpolarized, and the plateau was prolonged. The plateau could be changed by a polarizing current in DDT-containing seawater without K^+ in the same way as it could in seawater containing DDT. Thus it has become clear that the previous observation of the plateau formation in DDT media free of K^+ (*3*) was due to hyperpolarization by the removal of potassium.

Most of the voltage-clamp experiments were performed separately with normal axons and with axons that had been soaked in $5 \times 10^{-4}M$ DDT in seawater for a period of 40 minutes. Such separate experiments were necessary, because in most experiments with lobster axons an artificial node established by two sucrose streams did not survive beyond 20 to 30 minutes. The family of membrane currents associated with step depolarizations in a nor-