

The reaction of  $^{14}\text{CH}_2$  with  $\text{O}_2$ , while very efficient, forms  $^{14}\text{CO}$  and  $^{14}\text{CO}_2$  in equal amounts,<sup>8,9,12</sup> and adventitious  $\text{O}_2$  can thus be monitored through the detection of  $^{14}\text{CO}_2$ . For many of the samples in Table I, the  $^{14}\text{CO}_2$  yield was measured and shown to be very much less than that of  $^{14}\text{CO}$ , indicating that reaction with trace  $\text{O}_2$  was at most a minor source of  $^{14}\text{CO}$  in these experiments.

A possible oxirene intermediate of another type—formed by  $\text{CH}_2$  attack on  $\text{CH}_2\text{CO}$ —can be eliminated as the major source of  $^{14}\text{C}$  scrambling by the observation of appreciable  $^{14}\text{CO}$  in experiments with low percentages of ketene, e.g., 2% vs. 98% ethylene. In these low-ketene experiments, secondary attack on  $^{14}\text{CH}_2\text{CO}$  by photolytically produced radicals is essentially negligible. In pure ketene photolyses, however, approximately two molecules of ketene are removed for every molecule decomposed photolytically. Assuming that no  $^{14}\text{C}$  scrambling occurs in these radical attacks, we have calculated in Table I a per cent scrambling of  $^{14}\text{C}$  based on photolytically decomposed molecules alone.

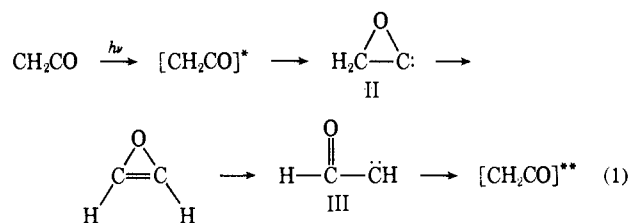
The quantum yield for ketene photodecomposition varies from about 0.1 at 3000 Torr to unity at the lowest pressures.<sup>2</sup> The quantum yield for dissociation with scrambling of  $^{14}\text{C}$  is thus about 70 times larger at low pressure than at high pressure. Although it cannot be conclusively proven, the scrambling of  $^{14}\text{C}$  position does not appear to come from a photoexcited state with a lifetime appreciably different from that exhibited by the bulk of the photoexcited ketene molecules.

The time dependence of the  $^{14}\text{CO}$  yields in two sets of comparable experiments at 3340 Å indicates that most of the reactions are single-photon processes, but the slow percentage increase of  $^{14}\text{CO}$  with time indicates that some  $^{14}\text{CH}_2\text{CO}$  molecules have rearranged without decomposition with the first photon, and are then subsequently decomposed by a second photon. We assume that this rearranged product is ketene itself, although nothing in our experiments rules out the possibility of its being some other rearranged  $\text{C}_2\text{H}_2\text{O}$  molecule—for example, stabilized oxirene itself. Since oxirene is unknown, its properties cannot be distinguished from those of ketene, and in our experiments, the properties of oxirene, if it exists as a stable molecule, would be expected to be essentially identical with those of ketene.

The scrambling of  $^{14}\text{C}$  prior to decomposition is excellent evidence for the existence of the symmetrical intermediate with equivalent carbon positions, as in I. The mechanism of formation of this intermediate is of course speculative, but could involve other intermediates such as the carbenes II and III. A set of plausible steps for this reaction includes each of these intermediates in succession, as shown in sequence 1. No evidence exists in our experiments for the presence of either II or III, and the observations could equally well be described by a concerted mechanism including the oxirene structure I at some stage in the reaction.

Since the  $\text{Hg}(^3\text{P}_1)$  photosensitization of ketene produces only 0.5%  $^{14}\text{C}$  scrambling, we postulate that an excited triplet state is not the precursor of the oxirene intermediate. The initial excited ketene  $(\text{CH}_2\text{CO})^*$  is probably an electronically excited singlet state, capable of the rearrangement to the cyclic carbene II, while the

final excited ketene  $(\text{CH}_2\text{CO})^{**}$  is probably the vibrationally excited ground state capable either of decomposition or stabilization. The stabilization path would then correspond to the radiationless pathway to ketene deexcitation.



The reactions shown in sequence 1 are not the only reaction paths available to excited ketene, for the per cent scrambling represents only a minor fraction of the total excited ketene species. The previous photolytic observations of oxirene intermediates have also shown that complete scrambling is not attained in those cases for which one of the substituents of the oxirene is an H atom.<sup>5,6</sup> These experiments involved two successive photolyses, the first causing a Wolff rearrangement and the second cleaving the substituted ketene formed in the first. Since oxirenes can now be expected in the second step, the question of oxirene participation in the Wolff rearrangement itself should be reexamined.

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### Structures of Stachysterone A, the First Natural 27-Carbon Steroid with a Rearranged Methyl Group, and Stachysterone B

Sir:

The bark of *Stachyurus praecox* SIEB. et ZUCC. ("kibushi" in Japanese) contains, in addition to  $\beta$ -ecdysone (1) (0.06% yield), four new phytoecdysones in yields of 0.002–0.004% each, which were designated stachysterones A (2), B (3), C (4), and D (5). All four have the molecular formula  $\text{C}_{27}\text{H}_{42}\text{O}_6$  corresponding to loss of water from  $\beta$ -ecdysone. Structures of stachysterones C and D have been reported recently.<sup>1</sup> The moulting activities of stachysterones A, B, and C are comparable to those of other ecdysones but stachysterone D is only weakly active. The following data enable one to assign structure 2 to stachysterone A and 3 to stachysterone B. Although more than 30 ecdysones have been characterized during the past 3 years,<sup>2</sup> stachysterone A is most unique in that: (i) it is the first naturally occurring  $\text{C}_{27}$  steroid with a rearranged methyl group; (ii) retention of its biological activity casts intriguing problems pertaining to structure–activity correlations; (iii) the cooccurrence of

(1) S. Imai, E. Murata, S. Fujioka, T. Matsuoka, M. Koreeda, and K. Nakanishi, *Chem. Commun.*, 352 (1970).

(2) K. Nakanishi, *Pure Appl. Chem.*, in press.

(12) R. L. Russell and F. S. Rowland, unpublished experiments.

$\beta$ -ecdysone and stachysterones A–D is of considerable biogenetic interest (see below).

Stachysterone A (amorphous) possessed the following constants:  $M^+$  at  $m/e$  462,  $M^+ - 2H_2O$  at  $m/e$  426.2699 (calcd for  $C_{27}H_{38}O_4$ : 426.2769); ir (KBr) 3400, 1640, 1590  $cm^{-1}$ ; uv (MeOH) 248 nm ( $\epsilon$  10,700); nmr ( $C_5D_5N$ ) 0.92 and 1.20 (s, 3 H, 10-Me and 14-Me),<sup>3</sup> 1.41 (s, 6 H, 25-Me's), 1.51 (s, 3 H, 20-Me), 3.85 (m, 1 H, 22-H), 4.09 (m, 1 H,  $W_{1/2} = 22$  Hz, 2-H), 4.31 (m, 1 H,  $W_{1/2} = 8$  Hz, 3-H), 5.98 (m, 1 H, 12-H), and 6.14 ppm (d, 1 H,  $J = 2.5$  Hz, 7-H); CD (dioxane)  $\Delta\epsilon_{338} -1.36$  and  $\Delta\epsilon_{354} -1.27$  (splitting due to fine structure,  $n \rightarrow \pi^*$ ).

Conspicuous and diagnostically valuable mass spectral peaks are depicted in structure 2. The fact that only two bn (nuclear fragment resulting from fission b upon electron impact; see Figure 1) peaks are present at  $m/e$  345 and 327 (differing in elements of water) is in contrast to the set of three bn peaks at  $m/e$  363, 345, and 327 present in spectra of common ecdysones with the 14 $\alpha$ -hydroxyl group.<sup>2,4</sup> This evidence together with the extra nmr olefinic multiplet at 5.98 ppm (12-H) indicated that the skeleton contains one extra trisubstituted double bond and that the 14 $\alpha$ -hydroxyl is absent. The latter point is ascertained by resistance of stachysterone A to HCl–MeOH, a treatment which in other ecdysones results in facile loss of 14 $\alpha$ -hydroxyl<sup>5</sup> (see below).

The ring A–B structure is identical with other ecdysones as indicated by the similarity of nmr peaks due to 2 $\alpha$ , 3 $\alpha$ , and 5 $\beta$  protons<sup>6</sup> in the 2,3,22-triacetate (amorphous):  $M^+ - H_2O$  at  $m/e$  570; ir (KBr) 3450, 1739, 1655, and 1245  $cm^{-1}$ ; nmr ( $CDCl_3$ ) 0.98 and 1.15 (s, 3 H, 10-Me and 14-Me), 1.20 (s, 6 H, 25-Me's), 1.30 (s, 3 H, 20-Me), 2.00, 2.10, and 2.22 (three acetoxy), 2.37 (dd, 1 H,  $J = 13$  and 5 Hz, 5-H), 4.90–5.10 (m, 2 H, 2- and 22-H), 5.35 (m, 1 H,  $W_{1/2} = 7.5$  Hz, 3-H), 5.93 (d, 1 H,  $J = 2.5$  Hz, 7-H), and 6.04 (m, 1 H, 12-H). The doublet nature of the 5.93-ppm signal indicates 14-C to be fully substituted. This fact together with the uniquely intense mass spectral peaks at  $m/e$  143 (96%) and 125 (34%) (see 2) (relative intensities of these peaks in other ecdysones are ca. 10%) establishes the 12-ene-14-methyl moiety. The 14-methyl group cannot adopt an equatorial conformation (molecular models); the  $\beta$  configuration is based on biogenetic considerations, especially in view of the cooccurrence of stachysterone B (3). The sign of the  $n \rightarrow \pi^*$  Cotton effect centered at 346 nm is negative and opposite to that of other ecdysones. In view of the known reliance of  $n \rightarrow \pi^*$  Cotton effect signs to the enone chirality,<sup>7</sup> this would indicate that the 6-one and 7-ene are twisted in a clockwise sense (*i.e.*, 6-one down).

(3) Nmr data are in parts per million relative to internal TMS. Abbreviations are: s, singlet; d, doublet; dd, doublet of doublets; m, multiplet; 3 H, three-proton intensity; 10-Me, methyl group attached to C-10;  $W_{1/2} = 8$  Hz, half-band width of 8 Hz;  $J = 2.5$  Hz, coupling constant of 2.5 Hz.

(4) Besides stachysterones A and B, only one other ecdysone lacking the 14 $\alpha$ -hydroxyl is known to date, *i.e.*, podectydysone B (8,14-dien-6-one): M. N. Galbraith, D. H. S. Horn, E. J. Middleton, and R. J. Hackney, *Chem. Commun.*, 402 (1969).

(5) P. Karlson, H. Hoffmeister, H. Hummel, P. Hocks, and G. Spittler, *Chem. Ber.*, 98, 2394 (1965).

(6) S. Imai, E. Murata, S. Fujioka, M. Koreeda, and K. Nakanishi, *Chem. Commun.*, 546 (1969).

(7) G. Sznatzke, *Tetrahedron*, 21, 439 (1965); M. Legrand and R. Viennet, *C. R. Acad. Sci., Paris*, 261, 1667 (1965); L. Velluz and M. Legrand, *Angew. Chem.*, 77, 842 (1965); K. Kuriyama, M. Moriyama, T. Iwata, and K. Tori, *Tetrahedron Lett.*, 1661 (1968).

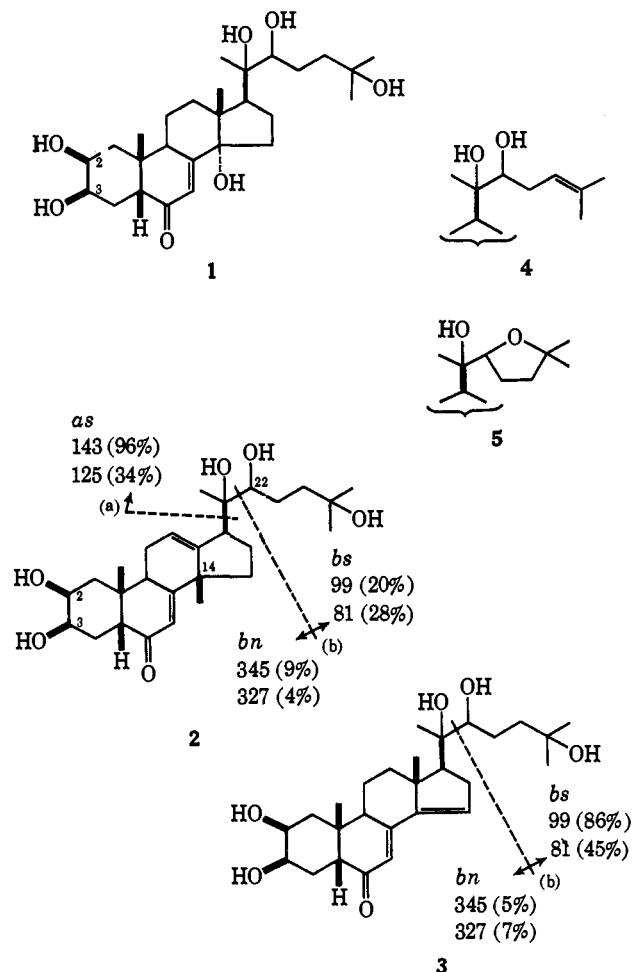


Figure 1. In structures 2 and 3, bn, bs indicate, respectively, nuclear and side-chain fragments resulting from fission b [ $C(20)-C(22)$ ] upon electron impact. Similarly an is the nuclear fragment resulting from fission a. Numerals denote mass units ( $m/e$ ) of mass spectral peaks, while percentages in parentheses are intensities relative to base peak at  $m/e$  43. (Skeletal structures of 4 and 5 are as in 1.)

Structure 3 is assigned to stachysterone B (amorphous) [ $M^+$  at  $m/e$  462,  $M^+ - H_2O$  at  $m/e$  444.2861 (calcd for  $C_{27}H_{40}O_5$ :  $m/e$  444.2815)] on the following evidence: ir (KBr) 3400, 1640, and 1590  $cm^{-1}$ ; uv (MeOH) 298 nm ( $\epsilon$  12,800); nmr ( $C_5D_5N$ ) 1.00 and 1.33 (s, 3 H, 10-Me and 13-Me), 1.42 (s, 6 H, 25-Me's), 1.49 (s, 3 H, 20-Me), 2.96 (dd, 1 H,  $J = 5$  and 13.5 Hz, 5-H), 3.76 (m, 1 H, 22-H), 4.82 (m, 1 H,  $W_{1/2} = 21$  Hz, 2-H), 4.40 (m,  $W_{1/2} = 7.5$  Hz, 3-H), 5.94 (dd, 1 H,  $J = 3$  and 3 Hz, 15-H), and 6.31 ppm (d, 1 H,  $J = 2.5$  Hz, 7-H); CD (dioxane)  $\Delta\epsilon_{288} -16.7$  and  $\Delta\epsilon_{355} -1.63$ . 2-,3,22-Triacetate (amorphous) showed the following characteristics:  $M^+$  at  $m/e$  588; ir (KBr) 3450, 1740, 1650, and 1595  $cm^{-1}$ ; nmr ( $CDCl_3$ ) 1.03 and 1.11 (s, 3 H, 10-Me and 13-Me), 1.21 (s, 6 H, 25-Me's), 1.30 (s, 3 H, 20-Me), 2.00 (s, 3 H) and 2.12 (s, 6 H, acetoxy), 4.86 (m, 1 H, 22-H), 5.01 (m, 1 H,  $W_{1/2} = 22$  Hz, 2-H), 5.32 (m, 1 H,  $W_{1/2} = 7.5$  Hz, 3-H), 6.00 (dd, 1 H,  $J = 3$  Hz, 15-H), and 6.11 ppm (d, 1 H,  $J = 2.5$  Hz, 7-H).

The side-chain structure and presence of the extra skeletal double bond is based on nmr and mass spectral data and the uv maximum at 298 nm. Structure 3 was established by conversion of ponasterone A<sup>8</sup> (1 without

(8) K. Nakanishi, M. Koreeda, S. Sasaki, M. L. Chang, and H. Y. Hsu, *Chem. Commun.*, 915 (1966).

25-OH) into the conjugated 7,14-dien-6-one (mp 167–168°) and deconjugated 8,14-dien-6-one (amorphous). Thus ponasterone A (500 mg) in MeOH–6 N HCl (2:3) was heated at 60° for 40 sec, the solution was poured into saturated aqueous sodium bicarbonate under ice cooling and extracted with ethyl acetate, and the extract was separated by preparative tlc to give 55 mg each of the dienones and 70 mg of starting material. Pertinent nmr signals of the 7,14-dien-6-one [CD (dioxane)  $\Delta\epsilon_{284}$  –16.0 and  $\Delta\epsilon_{355}$  –1.66] were similar to those of stachysterone B (excepting the 25-Me signals), while those of the 8,14-dien-6-one [CD (dioxane)  $\Delta\epsilon_{248}$  –4.8 and  $\Delta\epsilon_{294}$  –2.69] were similar to those reported for podedcdysone B.<sup>4</sup> The exceptionally large amplitudes of CD Cotton effects at ca. 285 nm ( $\pi \rightarrow \pi^*$ ) for stachysterone B and the corresponding dienone from ponasterone A ( $\Delta\epsilon$  –16.7 and –16.0) are to be noted.

Stachysterones A and B are not artifacts since they could also be isolated from the crude plant extract when this was passed through a column of silica gel, conditions under which ecdysones are not affected.

It is conceivable that in the plant  $\beta$ -ecdysone (1) is converted into stachysterone C (4) and then D (5), and also into stachysterone B (3) and then A (2). If 3 were the precursor of 2, it is interesting to note that migration of the 13-methyl to C-14 is the reverse of that encountered in the lanosterol biosynthesis. Could stachysterone A (2) be rearranged back to the normal ecdysone structure in insects to account for its activity? Not unrelated to this is that *Bombyx mori* is able to convert ponasterone A ([24,25-<sup>3</sup>H]) into  $\beta$ -ecdysone (and inokosterone), an observation which may explain the moulting activity of ponasterone A.<sup>9</sup>

(9) T. Okauchi, private communication.

(10) Insect Hormones. XIX. For part XVIII see ref 2.

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### Ajugalactone, an Insect Moulting Inhibitor as Tested by the *Chilo* Dipping Method

Sir:

An extensive effort to search for antiectdysones has resulted in the discovery<sup>1</sup> of several plants containing compounds which inhibit moulting of *Chilo suppressalis* (rice-stem borer) according to the dipping method.<sup>2</sup> We assign structure 1 to the first of such compounds, ajugalactone. The crude polyhydroxysteroid fraction<sup>3</sup> obtained from the methanol extract of 2 kg of the entire plant of *Ajuga decumbens* THUNB ("kiranso" in Japanese) was chromatographed on alumina and

(1) M. Goto, S. Imai, T. Toyosato, K. Otsuka, E. Murata, and K. Nakanishi, to be published.

(2) Y. Sato, M. Sakai, S. Imai, and S. Fujioka, *Appl. Ent. Zool. (Japan)*, 3, 49 (1968).

(3) S. Imai, S. Fujioka, K. Nakanishi, M. Koreeda, and T. Kurokawa, *Steroids*, 10, 557 (1967).

then silica gel, to afford in addition to the four phytoecdysones  $\beta$ -ecdysone (2.5 g), ajugasterone C<sup>4</sup> (30 mg), ajugasterone B<sup>5</sup> (30 mg), and cyasterone<sup>6</sup> (1.2 g), a new hydroxy steroid (200 mg) which exhibited unique activities. According to the *Chilo* dipping test, a 250–500-ppm solution of this compound completely suppressed the moulting activity of a 50-ppm solution on ponasterone A,<sup>7</sup> but it did not inactivate  $\beta$ -ecdysone. It is thus the first moulting hormone inhibitor to be found in nature,<sup>1</sup> although several synthetic 6-keto steroids (saturated) have been reported to possess antisclerotization effects on *Pyrrhocoris*.<sup>8</sup> However, the inhibition depends on the bioassay method<sup>9</sup> and the insect,<sup>10</sup> and until physiological aspects become clearer, the compounds cannot be called antiectdysones. A second natural inhibitor currently under study surprisingly has a totally different structure and antagonizes  $\beta$ -ecdysone but not ponasterone A (*Chilo* test).<sup>1</sup>

The physical constants of 1 and its diacetate<sup>2</sup> are as follows: ajugalactone (1), M<sup>+</sup> at 516.2751 (5%)<sup>9</sup> (calcd for C<sub>29</sub>H<sub>40</sub>O<sub>8</sub>: 516.2719); mp 225–235° dec; ir (KBr) 3440 (OH), 1718 (12-one), 1696 (lactone), 1654 cm<sup>-1</sup> (6-one); uv (MeOH) very broad peak centered around 233 nm ( $\epsilon$  15,700) ( $\pi \rightarrow \pi^*$  of en-6-one and unsaturated lactone); nmr (pyridine-*d*<sub>5</sub>) 0.74 (t, 3 H, *J* = 7 Hz, 28-Me),<sup>11</sup> 1.12 (s, 3 H, 10-Me), 1.49 and 1.65 (s, 3 H, 13-Me and 20-Me), 1.87 (s, 3 H, 25-Me), 3.00 (dd, 1 H, *J* = 4, 14 Hz, 5-H), 3.45 (ddd, 1 H, *J* = 2.5, 9.5, 9.5 Hz, 9-H), 4.00 (m, 1 H, *W*<sub>1/2</sub> = 20 Hz, 2-H), 4.16 (m, 1 H, *W*<sub>1/2</sub> = 7 Hz, 3-H), 4.42 (dd, 1 H, *J* = 5.5, 12 Hz, 22-H), and 6.33 (d, 1 H, *J* = 2.5 Hz, 7-H); CD (dioxane)  $\Delta\epsilon_{244}$  +11.56 ( $\pi \rightarrow \pi^*$  of 7-en-6-one and  $n \rightarrow \pi^*$  of unsaturated lactone),  $\Delta\epsilon_{288}$  –2.84 ( $n \rightarrow \pi^*$  of 12-one),  $\Delta\epsilon_{340}$  +0.86 ( $n \rightarrow \pi^*$  of 6-one); ajugalactone 2,3-diacetate (2), M<sup>+</sup> at 600; mp 278° dec; ir (CHCl<sub>3</sub>) 3450 (OH), 1740 (OAc), 1720 (12-one), 1710 (lactone), 1669 cm<sup>-1</sup> (6-one); nmr (acetone-*d*<sub>6</sub>) 1.09 (t, 3 H, *J* = 7 Hz, 28-Me), 1.17 (s, 3 H, 10-Me), 1.26 and 1.27 (s, 3 H, 13-Me and 20-Me), 1.83 (s, 3 H, 25-Me), 1.93 and 2.09 (s, 3 H, acetoxy), 2.34 (dd, 1 H, *J* = 7, 13 Hz, 5-H), 3.67 (ddd, 1 H, *J* = 3, 9.5, 9.5 Hz, 9-H), 4.18 (dd, 1 H, *J* = 6, 11 Hz, 22-H), 5.03 (ddd, 1 H, *J* = 3, 5, 12 Hz, 2-H), 5.30 (m, 1 H, *W*<sub>1/2</sub> = 6 Hz, 3-H), and 5.97 (d, 1 H, *J* = 3 Hz, 7-H).

The mass spectrum of ajugalactone (see 1) had a strong bs (side-chain fragment resulting from fission b upon electron impact; see Figure 1) peak at *m/e* 139, corresponding to 20–22 fission, and at *m/e* 111 (139 – CO);

(4) S. Imai, S. Fujioka, E. Murata, K. Otsuka, and K. Nakanishi, *Chem. Commun.*, 546 (1969).

(5) S. Imai, S. Fujioka, E. Murata, K. Otsuka, and K. Nakanishi, *ibid.*, 82 (1969).

(6) T. Takemoto, Y. Hikino, K. Nomoto, and H. Hikino, *Tetrahedron Lett.*, 3191 (1967).

(7) K. Nakanishi, M. Koreeda, S. Sasaki, M. L. Chang, and H. Y. Hsu, *Chem. Commun.*, 915 (1966).

(8) B. Režábová, J. Hora, V. Landa, V. Černý, and F. Šorm, *Steroids*, 11, 475 (1968); H. Velgova, L. Lábler, V. Černý, F. Šorm, and K. Sláma, *Collect. Czech. Chem. Commun.*, 33, 242 (1968).

(9) For example, injection of ajugalactone into diapausing pupae of *Manduca sexta* (tobacco horn-worm) resulted in ecdysone rather than antiectdysone action; private communication from Professor C. M. Williams, Harvard University, Cambridge, Mass.

(10) Private communication from Dr. K. Sláma, Institute of Entomology, Prague.

(11) Nmr data are in parts per million relative to internal TMS. Abbreviations are: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet; 3 H, three-proton intensity; 28-Me, methyl group attached to C-28; *W*<sub>1/2</sub> = 20 Hz, half-band width of 20 Hz; *J* = 7 Hz, coupling constant of 7 Hz.