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 podecdysone B.[4] 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 β-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-^{3}H]) into β-ecdysone (and inokosterone), an observation which may explain the moulting activity of ponasterone A.[9]

[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 antiecdysones has resulted in the discovery[1] of several plants containing compounds which inhibit moulting of *Chilo suppressalis* (rice-stem borer) according to the dipping method.[2] We assign structure **1** to the first of such compounds, ajugalactone. The crude polyhydroxysteroid fraction[3] obtained from the methanol extract of 2 kg of the entire plant of *Ajuga decumbens* THUNB ("kiranso" in Japanese) was chromatographed on alumina and then silica gel, to afford in addition to the four phytoecdysones β-ecdysone (2.5 g), ajugasterone C[4] (30 mg), ajugasterone B[5] (30 mg), and cyasterone[6] (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,[7] but it did not inactivate β-ecdysone. It is thus the first moulting hormone inhibitor to be found in nature,[1] although several synthetic 6-keto steroids (saturated) have been reported to possess antisclerotization effects on *Pyrrhocoris*.[8] However, the inhibition depends on the bioassay method[9] and the insect,[10] and until physiological aspects become clearer, the compounds cannot be called antiecdysones. A second natural inhibitor currently under study surprisingly has a totally different structure and antagonizes β-ecdysone but not ponasterone A (*Chilo* test).[1]

The physical constants of **1** and its diacetate[2] are as follows: ajugalactone (**1**), M$^+$ at 516.2751 (5%)[9] (calcd for $C_{29}H_{40}O_8$: 516.2719); mp 225–235° dec; ir (KBr) 3440 (OH), 1718 (12-one), 1696 (lactone), 1654 cm^{-1} (6-one); uv (MeOH) very broad peak centered around 233 nm (ϵ 15,700) ($\pi \rightarrow \pi^*$ of en-6-one and unsaturated lactone); nmr (pyridine-d_5) 0.74 (t, 3 H, J = 7 Hz, 28-Me),[11] 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, $W_{1/2}$ = 20 Hz, 2-H), 4.16 (m, 1 H, $W_{1/2}$ = 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$^+$ at 600; mp 278° dec; ir ($CHCl_3$) 3450 (OH), 1740 (OAc), 1720 (12-one), 1710 (lactone), 1669 cm^{-1} (6-one); nmr (acetone-d_6), 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, acetoxyls), 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, $W_{1/2}$ = 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);

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[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).

[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. Řežábová, J. Hora, V. Landa, V. Černý, and F. Šorm, *Steroids*, **11**, 475 (1968); H. Velgova, L. Labler, 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 antiecdysone 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; $W_{1/2}$ = 20 Hz, half-band width of 20 Hz; J = 7 Hz, coupling constant of 7 Hz.

a strong as (side-chain fragment from fission a) peak was present at *m/e* 183. The same set of peaks was also present in the acetate spectrum. These fragments are in full agreement with the side-chain structure indicated. The common ecdysones having partial structure **3** exhibit three prominent mass spectral peaks at *m/e* 363, 345, and 327 (bn) (nuclear fragment from fission b), but in ajugalactone the corresponding bn peaks appear at 14 higher mass units. Together with ir, uv, and CD data quoted above, this indicates that in contrast to the more than 30 ecdysones known to date,[12] an additional ketone group is present in the skeleton.

Similarity in the chemical shifts of protons attached to C-2, C-3, and C-5 in ajugalactone and the common ecdysones **3**, and the 9% increase in the 2-H peak intensity resulting from irradiation of 9α-H (due to intramolecular nuclear Overhauser effect, NOE)[13] in diacetate **2** establishes the configurations at C-2, C-3, and C-5, presence of the familiar 7-en-6-one moiety, and also indicates that ring A adopts a chair conformation (NOE).[13]

The extra ketone is six membered (ir of **2**) and therefore can only be at C-11 or C-12. Irradiation of the diacetate 3.67-ppm peak (9-H), which is coupled to the olefinic 7-H, converts a 2.61-ppm quartet (dd, J = 9.5 and 13 Hz, 11-H) to a doublet (J = 13 Hz); this shows that C-11 is a methylene having no adjacent protons other than the 9-H, *i.e.*, the ketone is at C-12. Two *tert*-hydroxyl groups are present. One is at C-14 as the splitting pattern of 7-H requires this carbon to be fully saturated, and also treatment of **1** with HCl–MeOH gives a compound absorbing at 294 nm, *i.e.*, presumably the 7,14-dien-6-one. The other hydroxyl group can only be at C-20. The side-chain structure is fully corroborated from nmr and other spectroscopic data.

The J value of the 22-H shows that the bond 20–22 is equatorially oriented with respect to the unsaturated δ-lactone. The absolute configuration shown in part structure **4** has been assigned to C-22 of a jaborasolactone A derivative[14] on the basis of its positive CD Cotton effect, $\Delta\epsilon$ +2.37 at 255 nm (n $\rightarrow$ π^*);[15] this C-22 configuration is identical with that of withaferin A (part structure **5**) derived from X-ray analysis.[16] As mentioned above, ajugalactone (**1**) has a positive Cotton effect around 244 nm, its very strong positive value of $\Delta\epsilon$ +11.56 being due to the overlap of two positive Cotton effects. Thus, a C-22 configuration similar to **4**, *i.e.*, *R*, can be assigned to ajugalactone provided the C-20 hydroxyl group exerts no sign-reversing effect; this configuration is identical with that of ponasterone A and most other ecdysones.[17]

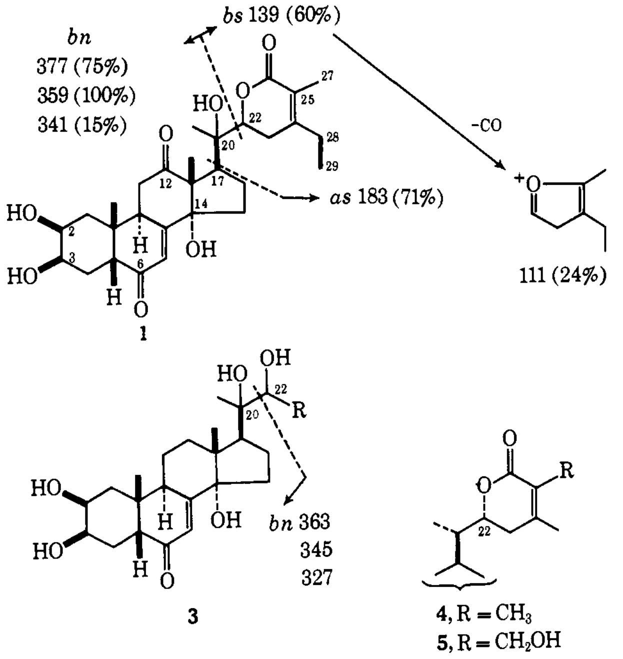

Figure 1. In structures **1** and **3**, bn, bs indicate, respectively, nuclear and side-chain fragments resulting from fission b [C(20)–C(22)] upon electron impact. Similarly an is the nuclear fragment resulting from fission a. Numerals denote mass units (*m/e*) of mass spectral peaks, while percentages in parentheses are intensities relative to base peak at *m/e* 359. The composition of all fragments indicated has been confirmed by exact mass measurements.

The 14-hydroxyl group has been assigned an α configuration on the basis of the isolation of ecdysones all having 14α-hydroxyls from the same plant. This is further supported by comparisons of chemical shifts of the 7-H (6.03 ppm in ajugalactone 2,3-diacetate, in $CDCl_3$) with an authentic 14β-hydroxy model. Thus, in $2\beta,3\beta,14\beta$-trihydroxy-23,24-bisnor-5α-chol-7-en-6-oic acid methyl ester 2,3-diacetate the 7-H peak appears at the low field of 6.57 ppm[18] due to near coplanarity of the hydroxyl group and the 7-H; in contrast, the 7-H in the 14α epimer is at 5.93 ppm (in $CDCl_3$).

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[12] K. Nakanishi, *Pure Appl. Chem.*, in press.

[13] M. Koreeda, N. Harada, and K. Nakanishi, *Chem. Commun.*, 548 (1969).

[14] Compound IXa in R. Tschesche, H. Schwang, H.-W. Fehlhaber, and G. Snatzke, *Tetrahedron*, **22**, 1129 (1966).

[15] G. Snatzke, H. Schwang, and P. Welzel in "Some Newer Physical Methods in Structural Chemistry," R. Bounett and T. G. Davis, Ed., United Trade Press Ltd., London, 1967, p 159.

[16] S. M. Kupchan, R. W. Doskotch, P. Bollinger, A. T. McPhail, G. A. Sim, and J. A. S. Renauld, *J. Amer. Chem. Soc.*, **87**, 5805 (1965); D. Lavie, E. Glotter, and Y. Shvo, *J. Chem. Soc.*, 7517 (1965).

[17] The C-22 configuration has been chemically established as being *R* by correlation with C-2 of (−)-L-leucine: M. Koreeda, R. Hagiwara, and K. Nakanishi, unpublished. The only ecdysone of established side-chain configuration is ecdysone itself, based on X-ray studies: R. Huber, and W. Hoppe, *Chem. Ber.*, **98**, 2403 (1965).

[18] We acknowledge Dr. J. B. Siddall, Zoecon Corp., for nmr data of the two 14-epimers.

[19] Insect Hormones. XX. For part XIX see S. Imai, E. Murata, S. Fujioka, T. Matsuoka, M. Koreeda, and K. Nakanishi, *J. Amer. Chem. Soc.*, **92**, 7510 (1970).

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Deuterium Isotope Effect on Competing Hydrogen and Carbomethoxy Migration in Benzotropilidene Photochemistry

Sir:

In an earlier report[1] we noted that photolysis of the benzonorcaradiene I yielded the rearranged isomer II in addition to fragmentation and hydrogen-shift

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[1] J. S. Swenton and A. J. Krubsack, *J. Amer. Chem. Soc.*, **91**, 786 (1969).