The Chemistry and Biochemistry of Insect Hormones

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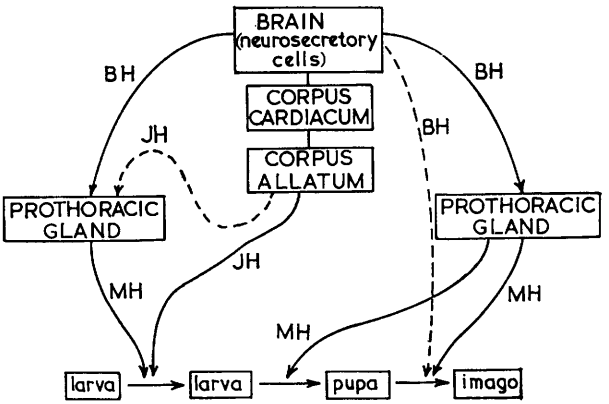
1 Introduction

Some 35 years ago the pioneering studies of Sir Vincent Wigglesworth[1] established the existence of insect hormones. Recent advances in the chemistry and biochemistry of these hormones have been particularly rapid and exciting, and it is with these developments that this Review will be primarily concerned. For the earlier literature the reader is directed to a number of fine texts.[2]

The objectives of the Review are twofold: to offer a current and critical view of the chemistry and biochemistry of insect hormones, and, since these events are seen through the eyes of a medicinally-oriented organic chemist, to develop the thesis that there is much to be learned from the insect that can be effectively translated to mammalian physiology and to other areas concerned with natural product chemistry.

The Review is limited to a consideration of Brain Hormone (BH), Juvenile Hormone (JH), and Moulting Hormone (MH), three metamorphosis hormones which play vital rôles in the regulation of post-embryonic development. Hormonal control of insect development may be schematically represented by the Figure.



Figure

[1] V. B. Wigglesworth, *Quart. J. micro. Sci.*, 1934, **77**, 191.
[2] (*a*) W. Etkin and L. I. Gilbert, 'Metamorphosis', Appleton-Century-Crofts, New York, 1968; (*b*) V. J. A. Novak, *Chem. Listy*, 1967, **61**, 340; (*c*) P. Karlson, *Rev. Pure Appl. Chem. (Australia)* 1967, **14**, 75.

2 Brain Hormone (BH)

A. Function.—Moulting in insects is initiated by neurosecretory cells of the brain which secrete a prothoracotropic hormone. The latter, commonly known as Brain (or Activation) Hormone, stimulates the prothoracic glands to release Moulting Hormone. It has been suggested[3] that in addition to its tropic action on the prothoracic gland, BH has a direct action on tissues, acting synergistically with ecdysone. The observation[4] that in the cockroach, *Periplaneta americana*, an elevation of the disaccharide trehalose (the principal circulatory carbohydrate in many insects) is produced by brain extracts (of *Periplaneta*) takes on new interest following Sacktor's very recent and exciting discovery[5] of the presence of the enzyme trehalase in mammalian tissues.

Although many other effects have been attributed to BH,[2b] a complete understanding of its action is still to be developed.

B. Chemistry.—In 1962, Kobayashi *et al.*[6] isolated 4 mg. of a crystalline substance by painstakingly dissecting 220,000 'brains' of the silkworm, *Bombyx mori*, and apparently demonstrated the hormonal activity of the extract. Subsequent studies[7] established unequivocally that the isolated material was cholesterol. That BH is purportedly cholesterol was in direct conflict with other published views. For example, Ischikawa and Ishizaki had previously[8] demonstrated that BH (similarly isolated from *Bombyx mori*) was water (and not ether) soluble. Based on its non-dialysable character, its response to typical protein precipitants, and its deactivation by proteolytic enzymes, it was subsequently concluded[9] that BH is a protein in nature.

Casting doubt on the probability of BH being cholesterol (cholesterol is a normal constituent of the insect diet and would, perhaps, not be expected to exert hormonal effects), Carlisle and Ellis[10] convincingly demonstrated that pure cholesterol had *no* effect on moulting in the locust, *Locusta migratoria migratorioides;* BH does induce moulting in this species.

A third view of the chemical nature of BH has been presented by Gersch.[11] From both the central nervous system and the cerebrum of the cockroach, *Periplaneta americana*, Gersch has been able to isolate the neurohormones C_1, D_1, C_2, and D_2. Only neurohormone D_1, a crystalline peptide, had demonstrable metamorphosis activity in the *Calliphora* assay. In his recent review Novak[2b] agrees that BH is a polypeptide, his support for Gersch's conclusion resting on the observation that known vertebrate-active peptides can affect the insect organism in a way similar to that of the insect neurohormones. In the

[3] M. Kobayashi, *Proc. Internat. Congr. Zoology*, 1963, **16**, 226.
[4] C. L. Ralph and R. McCarthy, *Nature*, 1964, **203**, 1195.
[5] See *The N.I.H. Record*, January 7, 1969.
[6] M. Kobayashi, J. Kirimura, and M. Saito, *Nature*, 1962, **195**, 516.
[7] J. Kirimura, M. Saito, and M. Kobayashi, *Nature*, 1962, **195**, 729.
[8] M. Ichikawa and H. Ishizaki, *Nature*, 1961, **191**, 933.
[9] M. Ichikawa and H. Ishizaki, *Nature*, 1963, **198**, 308.
[10] D. B. Carlisle and P. E. Ellis, *Nature*, 1963, **200** 496.
[11] M. Gersch, *Amer. Zoologist*, 1961, **1**, 53.

light of the wide variety of compounds capable of exerting Brain, Juvenile, and Moulting Hormone effects, the BH-activity of known polypeptides *per se* would seem to be slender supporting evidence for the chemical classification of BH.

C. BH-Like Substances.—Kobayashi[12] has claimed that in addition to cholesterol, a variety of steroids (progesterone, 7-dehydrocholesterol, campesterol) and certain 'physiologically vital' substances (thyroxin, noradrenaline) exert BH-activity. Among the inactives reported were gibberellin, adrenaline, and farnesol. Other workers claim[13] that farnesol (as well as farnesyl methyl ether and farnesyldiethylamine) does exert BH-activity, in addition to mimicking the action of Juvenile Hormone (see p. 378). Clearly, a generally acceptable bioassay for BH-activity must be available before the chemist can address himself to any elaboration of the BH molecule.

3 Juvenile Hormone (JH)

A. Function.—Juvenile Hormone (Neotenin) is secreted by the corpora allata; many functions have been attributed to it. The most striking property of JH is unquestionably its morphogenetic activity. JH prevents the metamorphosis of immature insects by acting in concert with Moulting Hormone to maintain the juvenile or larval character of the growing insect. It has also been demonstrated that the corpus allatum hormone can induce reversal of adult to larval integument.[14]

JH has a gonadotrophic effect in several orders of insects; it is necessary for yolk formation in the female and for full activity of the accessory glands in the male.[15] It has been suggested that JH controls ovarian and egg development by control of protein metabolism,[16] and that it may be indispensable for oocyte maturation because of its ability to enhance lipid synthesis in the ovary during oogenesis.[17]

An important effect of JH is its prothoracotropic action. In *Lepidoptera*, for example, JH activates the prothoracic gland.[15] It has been suggested[18] that the metabolic effects of JH are due to its action on prothoracic glands, since in the absence of the glands the hormone has no direct metabolic effects. Wigglewsorth[15] regards the so-called 'metabolic action' of JH with scepticism.

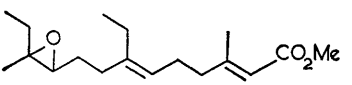
Allatectomy of the male desert locust, *Schistocerca gregaria*, leads to considerable accumulation of lipids, a condition which can be reversed by implantation of active corpora allata. Since allatectomy also results in loss of spontaneous motor activity, it is believed[19] that JH regulates the intensity of motor activity by a direct effect on the central nervous system. The accumulation of fat and

[12] M. Kobayashi, M. Saito, Y. Ishitoya, and N. Ikekawa, *Proc. Soc. Exp. Biol. Med.*, 1963, **114**, 316.
[13] A. Krishnakumaran and H. A. Schneiderman, *Amer. Zoologist*, 1963, **3**, 532.
[14] H. Röller and J. S. Bjerke, *Life Sci.*, 1965, **4**, 1617.
[15] V. B. Wigglesworth, *Nature*, 1965, **208**, 522.
[16] K. K. Thomas and J. L. Nation, *Biol. Bull*, 1966, **130**, 442.
[17] L. I. Gilbert, *Comp. Biochem. Physiol.*, 1967, **21**, 237.
[18] H. Oberlander and H. A. Schneiderman, *J. Insect Physiol.*, 1966, **12**, 37.
[19] T. R. Odhiambo, *J. Exp. Biol.*, 1966, **45**, 51, and references cited therein.

glycogen after allatectomy may thus be attributed to a decrease in spontaneous motor activity. Other instances of the influence of JH on behavioural patterns have been recorded,[15] although the possibility of feed-back effects from other organs should be recognised.

B. Chemistry.—In 1956, Williams[20] announced that the abdomen of the male silkmoth, *Platysamia cecropia* L., is an exceptionally rich source of JH. Noting that topical application of JH caused insects to die without completing their development, Williams recognised the powerful insecticidal potential of JH – powerful, since insects could scarcely develop resistance to their own hormones. It has been claimed that most mammalian tissues (including human placenta),[21] several micro-organisms,[22] and the parasite, *Nosema*,[23] all showed demonstrable JH-activity. Williams and Law[24] went on to purify the original *cecropia* extract some 50,000-fold, establishing that the major component of the material was the 9,10-epoxide of methyl hexadecanoate. Since synthetic (±)-*cis* and (±)-*trans* isomers were subsequently shown to be completely devoid of hormonal activity, it was concluded that JH was but a minor portion of the purified active fraction.

The impressive team work of Röller and his colleagues led first to a 10^5-fold purification of the *cecropia* extract,[14,25] and thence to the elaboration and synthesis of the JH molecule. By catalytic reduction (using 50 μg.), oxidative cleavage (15 μg.), and telling application of mass spectral analysis, the gross structure of JH was determined. From its n.m.r. spectrum (200 μg.) and subsequent synthesis[26] of the racemates of four of the possible sixteen stereoisomers, Röller *et al.* were able to refine their earlier structural conclusions and deduce that JH is methyl *trans,trans,cis*-10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate(1).[27]



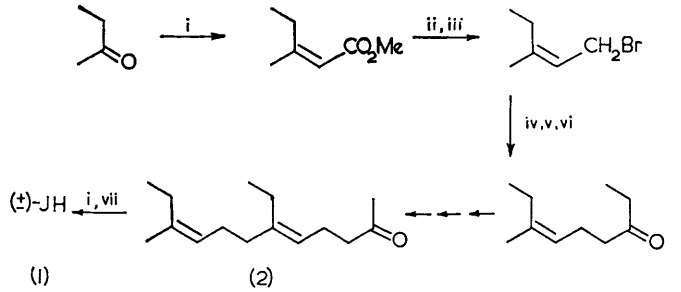
(I)

Racemic JH was subsequently pieced together[28] (Scheme 1) (overall yield: 0·05%) by successive application of the Wadsworth–Emmons reagent, the product being equipotent to the natural hormone.

The first of a series of stereospecific syntheses was a beautifully conceived route[29] to the Röller intermediate (2). The synthesis (Scheme 2) (overall yield: 4%) is based on sequential fragmentation of a bicyclic precursor (3), control of

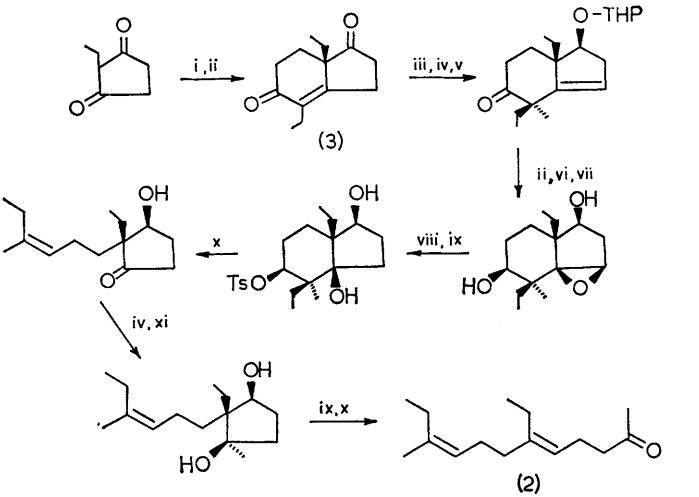
[20] C. M. Williams, *Nature*, 1956, **178**, 212.
[21] C. M. Williams, L. V. Moorhead, and J. F. Pullis, *Nature*, 1959, **183**, 405.
[22] H. A. Schneiderman, L. I. Gilbert, and M. J. Weinstein, *Nature*, 1960, **188**, 1041.
[23] F. M. Fisher jun. and R. C. Sanborn, *Nature*, 1962, **194**, 1193.
[24] C. M. Williams and J. H. Law, *J. Insect Physiol.*, 1965, **11**, 569.
[25] H. Röller, J. S. Bjerke, and W. H. McShan, *J. Insect Physiol.*, 1965, **11**, 1185.
[26] K. H. Dahm, H. Röller, and B. M. Trost, *Life Sci.*, 1968, **7**, 129.
[27] *Cf.* A. S. Meyer, H. A. Schneiderman, and E. Hanzmann, *Fed. Proc.*, 1968, **27**, 393.
[28] K. H. Dahm, B. M. Trost, and H. Röller, *J. Amer. Chem. Soc.*, 1967, **89**, 5292.
[29] R. Zurflüh, E. N. Wall, J. B. Siddall, and J. A. Edwards, *J. Amer. Chem. Soc.*, 1968, **90**, 6224.

olefin geometry therefore being transposed to a control of relative stereochemistry in cyclic systems.



Reagents: i, $(MeO)_2P(:O)\cdot CH_2\cdot CO_2Me$; ii, $LiAlH_4$; iii, PBr_3; iv, NaOEt–$MeCH_2\cdot COCH_2\cdot CO_2Et$; v, NaOH; vi, H^+; vii, *m*-chloroperbenzoic acid

Scheme 1

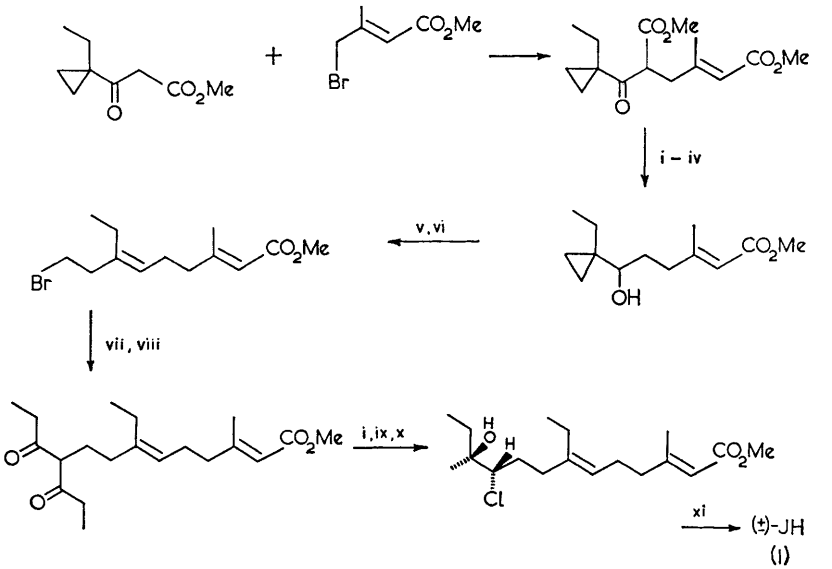


Reagents: i, Propylvinyl ketone; ii, H^+; iii, $NaBH_4$; iv, tetrahydropyranyl (THP) etherification; v, MeI; vi, $LiAl(OBu^t)_3H$; vii, *m*-chloroperbenzoic acid; viii, $LiAlH_4$; ix, tosylation; x, NaH; xi, MeLi.

Scheme 2

The 12-step Johnson synthesis[30] of racemic JH (Scheme 3) rests on Johnson's own stereoselective modification of the Julia method for introducing *trans*-trisubstituted double bonds, a step which involves the rearrangement of a cyclopropylcarbinyl to the homoallylic system.

[30] W. S. Johnson, T. Li, D. J. Faulkner, and S. F. Campbell, *J. Amer. Chem. Soc.*, 1968, **90**, 6225.

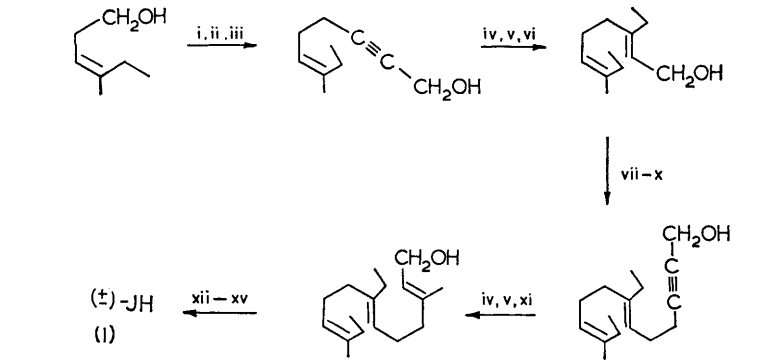


Reagents: i, $Ba(OH)_2$; ii, H^+; iii, CH_2N_2; iv, $NaBH_4$; v, PBr_3–LiBr; vi, $ZnBr_2$; vii, NaI, viii, Li enolate of heptane-3,5-dione; ix, $CuCl_2$–LiCl; x, MeMgCl; xi, K_2CO_3.

Scheme 3

While the overall yield is a more respectable 10% or so, the final product is only *ca.* 90% pure.

A third stereospecific total synthesis of (±)-JH, devised by Corey,[31] is again a fine example of imaginative chemistry. The route conceived embodies elegant application of no less than five novel synthetic processes initially introduced by Corey. The essence of the synthesis (overall yield 1%) is shown in Scheme 4.



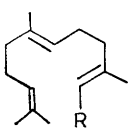
Reagents: i, TsCl; ii, LiC:CCH$_2$OTHP; iii, H^+; iv, $LiAlH_4$; v, I_2; vi, Et_2LiCu; vii, PBr_3; viii, $LiCH_2C{:}CSiMe_3$; ix, Ag^+ then CN^-; x, BuLi then CH_2O; xi, Me_2LiCu; xii, MnO_2; xiii, MeOH–NaCN; xiv, *N*-bromosuccinimide (NBS); xv, Pr^iO^-.

Scheme 4

[31] E. J. Corey, J. A. Katzenellenbogen, N. W. Gilman, S. A. Roman, and B. W. Erickson, *J. Amer. Chem. Soc.*, 1968, **90**, 5618.

Resolution of racemic JH, the biological evaluation of its optical antipodes, and determination of the absolute configuration of the natural hormone remain to be reported.

C. JH-Like Substances.—Historically, the first compounds with demonstrable JH-activity, farnesol (4; R = CH_2OH) and farnesal (4; R = CHO), were isolated by Schmialek[32] from the faeces of *Tenebrio molitor*.



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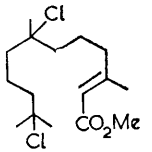


(5)

Evaluation of the JH-activity of a wide variety of farnesol derivatives, sesquiterpenoids in general, and both natural and synthetic compounds followed.[33] Among the more potent compounds were farnesyl methyl ether (4; R = CH_2OMe), farnesyldiethylamine (4; R = CH_2NEt_2), and dodecyl methyl ether. Synthetic methyl 10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate,[34] as a mixture of geometrical isomers, was 1600 times as active as farnesol.

In the course of their chemical studies on farnesol derivatives, Law, Williams *et al.*[35] described the synthesis of a neutral product of high JH-activity by treating farnesoic acid (4; R = CO_2H) with ethanolic HCl. The crude product, a mixture of at least six compounds, proved to be refractory to further purification. Emergence of the adult Yellow Fever mosquito, *Aedes aegypti*, is blocked by use of 1 part crude reaction product in 100,000 parts water, and lethal effects are exerted on the human body louse, *Pediculus humanus* var. *corporis*, the vector of epidemic typhus, trench fever, and epidemic relapsing fever.[36] Since the crude material is of obvious potential utility, it is to be hoped that further biological study follows the separation, purification, and identification of the active ingredient(s). The commercial availability of the Law–Williams mixture, dubbed 'Juvenile Hormone, Synthetic', does not, however, bode well for the purist.

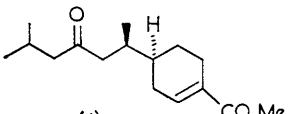
Šorm's group at the Czechoslovak Academy of Sciences more reasonably bubbled gaseous HCl through a solution of methyl farnesoate (4; R = CO_2Me) in methanol.[37] They obtained an almost quantitative yield of the dichloroester (5), found to be 10^5 times more active than farnesol when tested on the bug, *Pyrrhocoris apterus*. Sláma and his colleagues[38] have now described spectacular

[32] P. Schmialek, *Z. Naturforsch.*, 1961, **16***b*, 461.
[33] (*a*) H. Z. Levinson, *Riv. Parassit.*, 1966, **27**, 47; (*b*) A. Krishnakumaran and H. A. Schneiderman, *J. Insect Physiol.*, 1965, **11**, 1517, and references cited therein.
[34] W. S. Bowers, M. J. Thompson, and E. C. Uebel, *Life Sci.*, 1965, **4**, 2323.
[35] J. H. Law, C. Yuan, and C. M. Williams, *Proc. Nat. Acad. Sci. U.S.A.*, 1966, **55**, 576.
[36] J. W. Vinson and C. M. Williams, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **58**, 294, and references cited therein.
[37] M. Romaňuk, K. Sláma, and F. Šorm, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **57**, 349.
[38] P. Masner, K. Sláma, and V. Landa, *Nature*, 1968, **219**, 395, and references cited therein.

results with the dichloro-compound (5) which could have profound insecticidal significance. Their initial studies showed that as little as 1 μg. of the dichloro-ester (5) per specimen is enough to induce permanent sterility in the adult female of *Pyrrhocoris apterus*. These studies were extended to the treatment of males of *Pyrrhocoris*. When mated with ester-treated males, females laid completely sterile eggs for the remainder of their lives. Classically, in using the sterilised male technique to control a polygamous insect population, a female is still very likely to meet at least one healthy partner during her repeated matings. Sláma's technique is such that a treated male will render a female sterile no matter how many normal males she mates with subsequently. Other studies with the ester (5) suggest that it may have utility in preventing development of insect pests contaminating stored grain.[39]

A particularly fascinating chapter of JH-like compounds began when Sláma and Williams observed[40] that when the bug, *Pyrrhocoris apterus*, was transported from Prague to Boston, it failed to undergo normal metamorphosis; without exception all the animals died without completing metamorphosis. The source of JH-activity was eventually traced to exposure of the bugs to a specific brand of paper towel placed in the rearing jars. Following the evaluation of the JH-like activity of other samples of paper it was solemnly declared that *The New York Times*, *The Wall Street Journal*, and *Science* were active, whereas *The Times* and *Nature* were inactive! The active principle in American paper, the so-called 'Paper Factor', originated in the balsam fir, *Abies balsamea*. It was postulated that such trees had evolved an extremely sophisticated defensive system against insect predators sharing the endocrine sensitivities of *P. apterus*. Based on their examination of deformed specimens of *P. apterus*, Carlisle *et al.*[41] preferred to regard the 'Paper Factor' as inducing a 'pathological growth pattern' rather than JH-like effects.

With *Abies balsamea* as their source, Bowers and his colleagues[42] successfully isolated, purified, and characterised the active principle (+)-juvabione, as the methyl ester of todomatuic acid (6).



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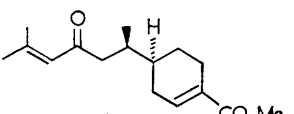


(7)

From balsam fir indigenous to Czechoslovakia, Černý and his co-workers[43] isolated two active constituents, (+)-juvabione (6), and the slightly less active

[39] J.P. Thomas, and P. L. Bhatnagar-Thomas, *Nature*, 1968, **219**, 949.
[40] C. M. Williams and K. Sláma, *Biol. Bull.*, 1966, **130**, 247, and references cited therein.
[41] D. B. Carlisle, P. E. Ellis, Z. Brettschneiderova, and V. J. A. Novák, *J. Endocrinol.*, 1966, **35**, 211.
[42] W. S. Bowers, H. M. Fales, M. J. Thompson, and E. C. Uebel, *Science*, 1966, **154**, 1020.
[43] V. Černý, L. Dolejš, L. Lábler, F. Šorm, and K. Sláma, *Coll. Czech. Chem. Comm.*, 1967, **32**, 3926.

(+)-dehydrojuvabione (7). We now wait to learn more about other active constituents present in Czechoslovakian balsam fir. Biogenetic considerations would suggest that these could be isomers of dehydrojuvabione, compounds at higher levels of oxidation, or even bicylic sesquiterpenoids.

Mori and Matsui were the first to describe syntheses of (±)-juvabione and (±)-dehydrojuvabione and to compare biologically (±)-juvabione with the natural dextrorotatory product.[44] Ayyar and Rao[45] subsequently carried out a basically similar synthesis of (±)-juvabione. More recent reports[46] describe stereospecific syntheses of natural (+)-juvabione and assignment of the *R*-configuration of the two asymmetric centres. Extending their interest in juvabione chemistry to related structures of possible JH-interest, the Prague group has now prepared[47] highly active analogues wherein the cyclohexane moiety is fully aromatised.

A most interesting observation[48] is that a number of non-sesquiterpenoidal synergists (used to enhance the insecticidal effects of pyrethrins and carbamates) possess *per se* significant JH-activity. Bowers[48] speculates that since the JH-active piperonyl butoxide and sesoxane act synergistically by inhibiting microsomal oxidation and hydroxylation enzymes, other JH-active substances could exert their effects by regulating these or similar enzymes.

From their extensive studies on JH and related compounds, Röller and his colleagues,[49] Wigglesworth,[50] Meyer,[27] and others have looked for meaningful structure–activity correlations. No clear relationship has yet been established. Noteworthy, however, is the fact that the homologous ethyl ester of JH shows eight times the morphogenetic activity (in *Tenebrio molitor*) of natural JH; his is the most potent JH-active compound yet described.

4 Moulting Hormone

A. Function.—A third insect hormone governing metamorphosis is Moulting Hormone (MH). It is produced in the prothoracic (or ecdysial) gland by stimulation of BH. It has been referred to variously and descriptively as 'Pupation Hormone', 'Growth Hormone', 'Growth and Differentiation Hormone', 'Metamorphosis Hormone', and 'Prothoracic Gland Hormone'. In its pure crystalline form MH is now referred to as ecdysone. During larval development MH and JH are both secreted thus initiating larval moulting. Transformation to the pupa occurs when JH levels are low enough and MH is secreted alone. Imaginal development is controlled by MH, possibly in synergy with BH.

A number of different actions have been attributed to MH. The most dramatic is its ability to induce protein synthesis. Fifteen minutes after injecting as little

[44] K. Mori and M. Matsui, *Tetrahedron*, 1968, **24**, 3127, and references cited therein.
[45] K. Subrahmania Ayyar and G. S. Krishna Rao, *Canad. J. Chem.*, 1968, **46**, 1467.
[46] (*a*) A. J. Birch, P. L. Macdonald, and V. H. Powell, *Tetrahedron Letters*, 1969, 351; (*b*) B. A. Pawson, H.-C. Cheung, S. Gurbaxani, and G. Saucy, *Chem. Comm.*, 1968, 1057.
[47] M. Suchý, K. Sláma, and F. Šorm, *Science*, 1968, **162**, 582.
[48] W. S. Bowers, *Science*, 1968, **161**, 895.
[49] H. Röller, and K. H. Dahm, *Recent Progr. Hormone Res.*, 1968, **24**, 651.
[50] V. B. Wigglesworth, *Nature*, 1969, **221**, 190.

as 2×10^{-6} μg. ecdysone into the midge, *Chironomus tentans*, characteristic 'puffing' of the giant salivary gland chromosomes occurs.[51] The 'puffs', indicating gene activation, are sites of messenger RNA synthesis. The short time and small dose needed to produce the effect suggest that chromosomal 'puffing' is a primary reaction. Although it is conceivable that hormone-induced 'puffing' reflects direct interaction of ecdysone with DNA, Karlson[52] prefers to view ecdysone as combining with chromosomal protein, possibly a histone. Clever[53] sees the primary steps in the cellular response to ecdysone as follows:

$$\text{Ecdysone} \rightarrow (?) \rightarrow \underset{\text{Specific Genes}}{\text{Activation of}} \rightarrow \text{m-RNA}$$

$$\downarrow$$

$$\text{Further Gene Activation} \rightleftharpoons (?) \leftarrow \text{Protein}$$

A molecular basis of learning and memory is now attributed to quantitative changes in RNA and protein synthesis, and compounds capable of inducing RNA synthesis are of direct interest in this regard.[54] It would, therefore, seem worthwhile to examine ecdysone for such properties, especially in vertebrate systems, although some early results[55] should be noted: on injecting prothoracic gland extracts into locusts, the time devoted to marching (a response which is not learned) was reduced, whilst no apparent effect was observed on social aggregation (a learned activity).

The view that ecdysone acts directly on the gene is not shared by Kroeger.[56] Observing that in explanted salivary glands of *Chironomus thummi* 'puffs' can be induced by high K^+ concentrations, Kroeger concludes that gene activity is controlled by the $Na^+ : K^+$ ratio in the nuclear sap and that ecdysone controls the ion balance. Kroeger's hypothesis has been rejected by some workers[52] but supported by others.[57] Available evidence would suggest that the case for direct action is 'not proven'.

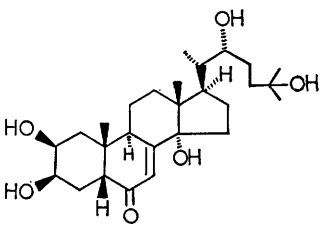
Dopa [β-(3,4-dihydroxyphenyl)-L-alanine] decarboxylase, an important mammalian enzyme, plays a critical rôle in the production of the sclerotising agent *N*-acetyldopamine. Injection of ecdysone into *Calliphora erythrocephala* larvae results in an increase in dopa decarboxylase activity.[51] It now seems likely that in *Calliphora*, a specific 5-hydroxytryptophan (5-HTP) decarboxylase exists and, further, this new enzyme is repressed by ecdysone.[58] Dopa decarboxylase had previously been thought to effect the decarboxylation of 5-HTP (to serotonin).

[51] P. Karlson and C. E. Sekeris, *Recent Progr. Hormone Res.*, 1966, **22**, 473, and references cited therein.
[52] P. Karlson and C. E. Sekeris, *Acta Endocrinol.*, 1966, **53**, 505.
[53] U. Clever and C. G. Romball, *Proc. Nat. Acad. Sci. U.S.A.*, 1966, **56**, 1470.
[54] J. Gaito, in 'Chemistry and Learning', ed. W. C. Corning and S. C. Ratner, Plenum Press, New York 1967, p. 23.
[55] D. B. Carlisle and P. E. Ellis, *Nature*, 1963, **200**, 603.
[56] H. Kroeger and M. Lezzi, *Ann. Rev. Entomol.*, 1966, **11**, 1, and references cited therein.
[57] O. Hechter and I. D. K. Halkerston, *Ann. Rev. Physiol.*, 1965, **27**, 133.
[58] V. J. Marmaras, C. E. Sekeris, and P. Karlson, *Acta Biochim. Polon.*, 1966, **13**, 305.

Kobayashi[59] has shown that on injection of ecdysone into brainless pupae of *Bombyx mori*, labelled glucose is preferentially converted to the disaccharide trehalose at the expense of the polysaccharide glycogen. It is therefore concluded that ecdysone affects the enzymes governing trehalose *versus* glycogen biosynthesis.

In vivo experiments using mice indicate[60] some effectiveness of ecdysone in causing regression and inhibition of sarcoma 180 tumours. The absence of MH-active material in normal mammalian tissue (specifically man) was also noted.[60]

B. Chemistry.—The isolation and characterisation of MH was known to be a Herculean task at the outset. Accepting the challenge, Butenandt and Karlson[61] reported in 1954 the isolation of 25 mg. of the crystalline hormone ecdysone (hitherto α-ecdysone) from 500 kg. of the pupae of the commercially used silkworm, *Bombyx mori*. With the name of Karlson pre-eminent, a series of outstanding papers devoted to the chemistry of ecdysone followed, culminating in a definitive *X*-ray crystallographic analysis.[62] The complete structure was thus elucidated, showing ecdysone to be the steroid 2β,3β,14α,22*R*,25-pentahydroxy-Δ^7-5β-cholesten-6-one (8).

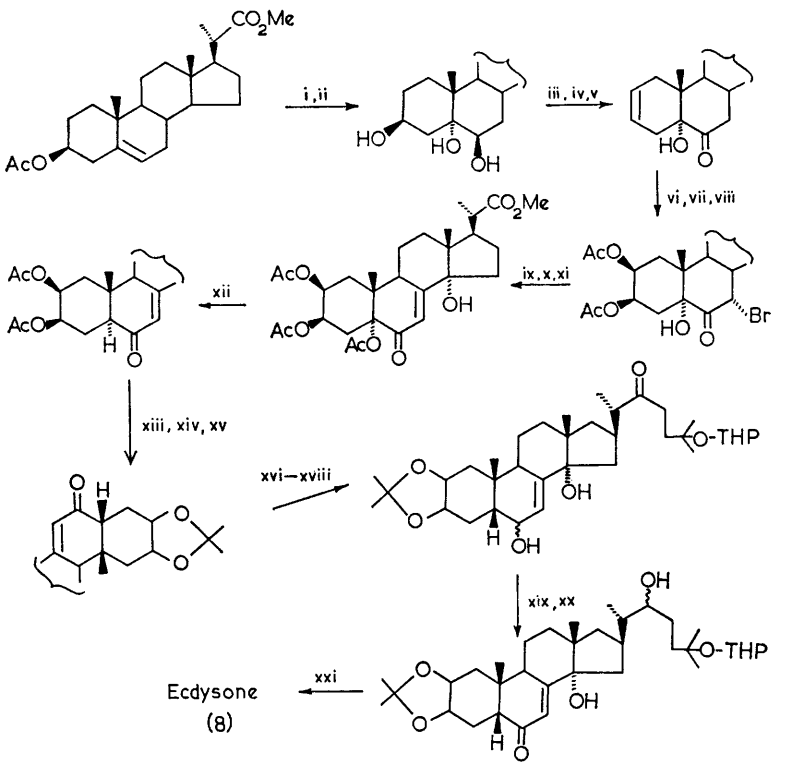


(8)

Confirmation of the structure of ecdysone (8) by unambiguous, straightforward synthesis was announced almost simultaneously by two groups, Syntex[63] and Schering AG–Hofmann-La Roche.[64] Outlines of these syntheses (Schemes 5 and 6 respectively), both of which start with bisnorcholenic acid derivatives, are shown.

A second, more economic synthesis subsequently devised by the Schering–Roche group[65] takes advantage of the availability of ergosterol. Stigmasterol

[59] M. Kobayashi and S. Kimura, *J. Insect Physiol.*, 1967, **13**, 545.
[60] W. J. Burdette, *Acta, Unio Internat. Contra Cancrum*, 1964, **20**, 1531.
[61] A. Butenandt and P. Karlson, *Z. Naturforsch.*, 1954, **9b**, 389.
[62] R. Huber and W. Hoppe, *Chem. Ber.*, 1965, **98**, 2403.
[63] J. B. Siddall, A. D. Cross, and J. H. Fried, *J. Amer. Chem. Soc.*, 1966, **88**, 862, and references cited therein.
[64] U. Kerb, G. Schultz, P. Hocks, R. Wiechert, A. Furlenmeier, A. Fürst, A. Langemann, and G. Waldvogel, *Helv. Chim. Acta.*, 1966, **49**, 1601, and references cited therein.
[65] A. Furlenmeier, A. Fürst, A. Langemann, G. Waldvogel, P. Hocks, U. Kerb, and R. Wiechert, *Helv. Chim. Acta*, 1967, **50**, 2387.



Reagents: i, HCO_2H–H_2O_2; ii, NaOH; iii, NBS; iv, tosylation; v, Li_2CO_3; vi, AgOAc–I_2–AcOH; vii, AcO_2–py; viii, Br_2–HBr; ix, —HBr; x, Ac_2O–H^+; xi, SeO_2; xii, $CrCl_2$; xiii, hydrolysis; xiv, acetone–TsOH; xv, K_2CO_3; xvi, $LiAl(OBu^t)_3H$; xvii, Li salt of $PhSO(CH_2)_2CMe_2OTHP$; xviii, Al–Hg; xix, $LiAlH_4$; xx, MnO_2; xxi, H^+.

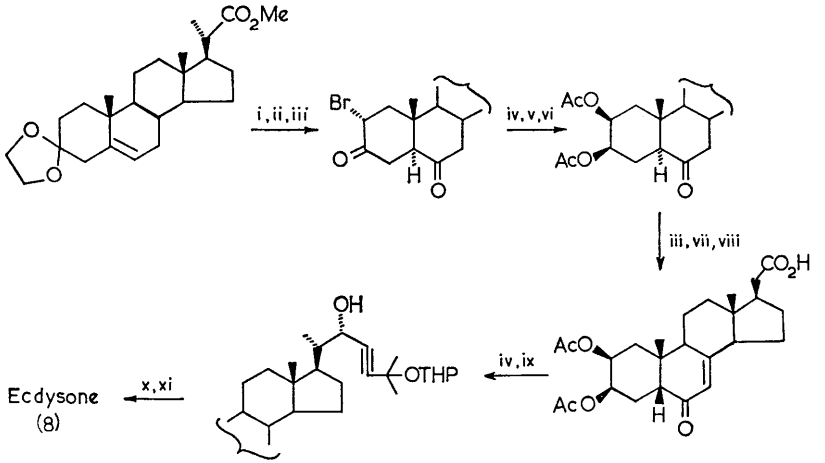
Scheme 5

was the readily available starting material used by Mori *et al.*[66] in their successful synthetic efforts.

Schering's disclosed[67] central nervous system effects for a variety of their synthetic 2,3-dihydroxycholestane derivatives bring to mind the earlier claim,[68] subsequently denied,[69] of potent analgetic activity of certain polyalkoxyestratrienes typified by 2,3,4-trimethoxyoestra-1,3,5(10)-triene-17β-ol.

Novel methods for the introduction of the 14α-hydroxy-substituent appear to be a point of continuing interest. For example, photosensitised oxidation of $\Delta^{8(13)}$-6-ketosteroids yields the corresponding Δ^{7}-14α-hydroperoxides; reduc-

[66] H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull. Japan*, 1968, **16**, 563.
[67] See for example, Schering AG., S. Afr. P. 1862/1967.
[68] L. R. Axelrod, P. N. Rao, and D. H. Baeder, *J. Amer. Chem. Soc.*, 1966, **88**, 856.
[69] D. R. VanDeripe, G. B. Hoey, W. R. Teeters, and T. W. Tusing, *J. Amer. Chem. Soc.*, 1966, **88**, 5366.



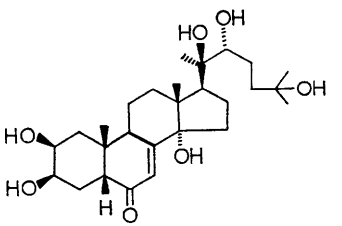
Reagents: i, epoxidation; ii, H^+; iii, Br_2; iv, $LiAl(OBu^t)_3H$; v, Ac_2O; vi, AgOAc; vii, —HBr; viii, LiI; ix, $BrMgC{:}CMe_2OTHP$; x, H_2–PtO_2; xi, SeO_2.

Scheme 6

tion of the latter afford the 14α-hydroxy-derivatives in near quantitative overall yield.[70] Microbiological insertion of a 14α-hydroxy-group into 6-keto-Δ^7-steroidal substrates has also been claimed.[71]

C. MH-Like Substances.—A number of naturally occurring MH-active compounds have now been isolated and characterised. To date, these compounds are all steroids structurally related to the ecdysone molecule. Such has been the furious pace of discovery, that the synonymity of compounds under study by different groups frequently goes unrecognised.

Following the structural elucidation of ecdysone, a second major development in hormone research was the isolation of 2 mg. of pure crustecdysone (9) from one ton of the crayfish, *Jasus lalandei*.[72] The hormone is responsible for crustacean



(9)

crustecdysone
β-ecdysone
ecdysterone
20-hydroxyecdysone
polypodine A
iso-inokosterone

[70] N. Furutachi, Y. Nakadaira, and K. Nakanishi, *Chem. Comm.*, 1968, 1625; *cf.* Syntex Corp., Fr. P. 1,524,924/1968.
[71] Schering AG., Eire P. 726/1965.
[72] D. H. S. Horn, E. J. Middleton, J. A. Wunderlich, and F. Hampshire, *Chem. Comm.*, 1966, 339, and references cited therein.

ecdysis but is also highly active in the *Calliphora* bioassay.

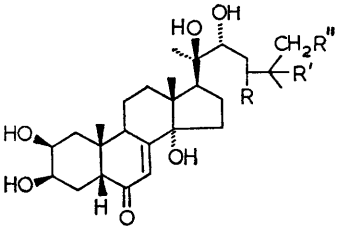
Recognising that crustecdysone is identical with β-ecdysone,[73] a second MH-active compound present in *Bombyx mori* pupae, Horn[72] postulated that ecdysone is the biosynthetic precursor of crustecdysone and that the two compounds may act in concert to effect different stages of the moulting process. An alternative view is that crustecdysone is simply an independently active (but less active) metabolite of ecdysone. The further suggestion[72] that hydroxylation of ecydsone at C–20 could be a preliminary step to C–20—C–22 bond fission to a pregnenolone now seems unlikely.[74] It now appears that structure (9) is synonymously crustecdysone,[72] β-ecdysone,[73] ecdysterone,[75] 20-hydroxyecdysone,[75] polypodine A,[76] and iso-inokosterone.[77] The synthesis of ecdysterone has been achieved.[78]

From a staggering 3 tons of crayfish waste, Horn *et al.*[79] more recently reported the successful isolation of 200 μg. of a deoxycrustecdysone now believed to be the 2-deoxy-derivative. In addition to ecdysone and ecdysterone a third MH-active substance, 20,26-dihydroxyecdysone, has been isolated from the tobacco hornworm, *Manduca sexta*.[80]

A new dimension in natural-product chemistry was created by the discovery of MH-like substances in plants. Consistent with Nakanishi's observation[81] that *Podocarpus nakaii* contains crustecdysone-related compounds, the wood of *Podocarpus elatus* R.Br., an Australian timber tree, was reported[82] to be a 'rich source' of crustecdysone. Thus, for the first time, steroids with MH-activity became readily available for further chemical and biological study. That *P. elatus* was known to be particularly resistant to insect attack prompted the suggestion[82] that the plant elaborates the hormone to interfere with the growth processes of insect predators.

Following Nakanishi's initial discovery, many MH-active phytosteroids have been and continue to be isolated and characterised. From 4·8 kg. of dried leaves of *Podocarpus nakaii* Hay one of the four active constituents isolated[81] was crystalline ponasterone A (2 g.). Its structure was shown to be (10; R = R′ = R″ = H).

[73] P. Hocks, G. Schulz, and P. Karlson, *Naturwiss.*, 1967, **54**, 44, and references cited therein.
[74] J. B. Siddall, D. H. S. Horn, and E. J. Middleton, *Chem. Comm.*, 1967, 899.
[75] M. N. Galbraith, D. H. S. Horn, P. Hocks, G. Schulz, and H. Hoffmeister, *Naturwiss.*, 1967, 471, and references cited therein.
[76] J. Jizba, V. Herout, and F. Sorm, *Tetrahedron Letters*, 1967, 1689.
[77] T. Takemoto, S. Ogawa, N. Nishimoto, and H. Hoffmeister, *Z. Naturforsch.*, 1967, **22b**, 681.
[78] (*a*) U. Kerb, R. Wiechert, A. Furlenmeier, and A. Fürst, *Tetrahedron Letters*, 1968, 4277; (*b*) G. Hüppi and J. B. Siddall, *J. Amer. Chem. Soc.*, 1967, **89**, 6790.
[79] M. N. Galbraith, D. H. S. Horn, E. J. Middleton, and R. J. Hackney, *Chem. Comm.*, 1968, 83.
[80] M. J. Thompson, J. N. Kaplanis, W. E. Robbins, and R. T. Yamamoto, *Chem. Comm.*, 1967, 650.
[81] K. Nakanishi, M. Koreeda, S. Sasaki, M. L. Chang, and H. Y. Hsu, *Chem. Comm.*, 1966, 915.
[82] M. N. Galbraith and D. H. S. Horn, *Chem. Comm.*, 1966, 905.



(10)

The MH-active 3β-glycoside of ponasterone A was recently isolated from *Pteridium aquilinum* var. *latiusculum*.[83] Initially called warabisterone, it now bears the name ponasteroside A.

The root of *Achyranthes fauriei*, a common and ubiquitous Japanese herb, has been especially well studied by Takemoto and his colleagues.[84] It has long been used in Japanese folk medicine as an emmenagogue, analgetic, and diuretic. Two steroidal MH-active compounds have been isolated, iso-inokosterone (ecdysterone) (9) and inokosterone (10; R = R′ = H, R″ = OH). It now seems probable[85] that callinecdysone A, extracted from the crab, *Callinectes sapidus*, is either one of the inokosterone isomers (25*R* or 25*S*) or, like inokosterone, an epimeric mixture of the two.

A second heptahydroxycoprostenone derivative, polypodine B, has been described.[86] Initially isolated from *Polypodium vulgare* L.,[86a] and then, together with viticosterone E (25-acetoxyecdysterone), from *Vitex megapotamica*,[86b] it is four times as active as ecdysone in the *Calliphora* bioassay, and is the most active MH-like substance yet described. Of unknown stereochemistry at C–20 and C–22, its salient structural interest lies in the presence of a 5β-OH grouping.

On routine screening of plant materials,[87] extracts of the roots of *Cyathula capitata* were found to have MH-activity. Four biologically active C_{29} constituents have now been characterised:[88] amarasterone A (10; R = Et, R′ = H, R″ = OH), amarasterone B (10; R = CH_2CH_2OH, R′ = R″ = H), and two side-chain lactone derivatives, cyasterone and capitasterone. A fifth C_{29} MH-active steroid, lemmasterone (10; R = Et, R′ = OH, R″ = H) has been isolated[89] from *Lemmaphyllum microphyllum*. It would seem that podecydsone

[83] T. Takemoto, S. Arihara, and H. Hikino, *Tetrahedron Letters*, 1968, 4199.
[84] T. Takemoto, Y. Hikino, S. Arihara, H. Hikino, S. Ogawa, and N. Nishimoto, *Tetrahedron Letters*, 1968, 2475, and references cited therein.
[85] A. Faux, D. H. S. Horn, E. J. Middleton, H. M. Fales, and M. E. Lowe, *Chem. Comm.*, 1969, 175.
[86] (*a*) J. Jizba, V. Herout, and F. Sorm, *Tetrahedron Letters*, 1967, 5139; (*b*) H. Rimpler, *Tetrahedron Letters*, 1969, 329.
[87] T. Takemoto, S. Ogawa, N. Nishimoto, S. Arihara, and K. Bue, *J. Pharm. Soc. Japan*, 1967, **87**, 1414.
[88] T. Takemoto, K. Nomoto, and H. Hikino, *Tetrahedron Letters*, 1968, 4953, and references cited therein.
[89] T. Takemoto, Y. Hikino, T. Arai, and H. Hikino, *Tetrahedron Letters*, 1968, 4061.

A[90] and makisterone C[91] are identical with lemmasterone. In addition to makisterone C, Imai and his co-workers[91] describe three other MH-active steroids from *Podocarpus macrophyllus:* a second C_{29} steroid, makisterone D (10; R = $CHOHCH_3$, R′ = R″ = H), and two C_{28} derivatives, makisterone A (10; R = Et, R′ = OH, R″ = H), and makisterone B (10; R = Et, R′ = H, R″ = OH). Callinecdysone B[85] is now believed to be either makisterone A or its C_{24} isomer. Imai *et al.*[92] subsequently preface their introduction of ajugasterone B, a Δ^{25} derivative of (10; R = Et, R′ = H, R″ = OH) by the pregnant statement that 1056 species of Japanese plants, representing 186 families, are under evaluation for MH-active constituents!

The described[88] periodate cleavage of cyasterone [and ecdysterone (9)] to yield, *via* a seco dialdehyde, an A-norprogesterone derivative could seemingly be a useful source of starting material for studies directed towards anti-androgenic agents.[93]

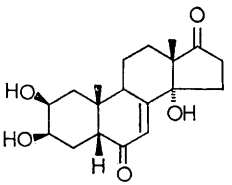
Pterosterone (10; R = OH, R′ = R″ = H), isolated from *Lastrea thelypteris*,[86b,94] is a stereoisomer of the 2α,3α-dihydroxy-derivative, ponasterone C, extracted from *Podocarpus nakaii*.[81] Shidasterone (10; R = R″ = H, R′ = OH), isolated from *Blechnum niponicum*,[95] is seemingly a stereoisomer of ecdysterone but is not, as was once thought the C_{22}-epimer.

Of mammalian significance is the observation[96] that pterosterone, cyasterone, inokosterone, ponasterone A, and ecdysterone are as potent as the anabolic steroid 4-chlorotestosterone in stimulating protein synthesis in the mouse.

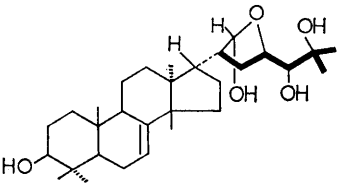
A departure from the trend of isolable phytosteroids of type (10) comes with the characterisation of rubrosterone (11).[97] The 17-keto-derivative, which shows little MH-activity, is a suggested metabolite of ecdysterone (9) and inokosterone (10; R = R′ = H, R″ = OH); all three compounds are present in *Achyranthes rubrofusca.*

A number of purely synthetic ecdysone derivatives capable of exerting hormonomimetic effects have been described.[98] Future syntheses will presumably take advantage of the commercial availability of ecdysterone and inokosterone, and, in this regard, the introduction[99] of a trimethylsilyl ether as a protecting

[90] M. N. Galbraith, D. H. S. Horn, Q. N. Porter, and R. J. Hackney, *Chem. Comm.*, 1968, 971.
[91] S. Imai, S. Fujioka, E. Murata, Y. Sasakawa, and K. Nakanishi, *Tetrahedron Letters*, 1968, 3887.
[92] S. Imai, S. Fujioka, E. Murata, K. Otsuka, and K. Nakanishi, *Chem. Comm.*, 1969, 82.
[93] See for example, L. J. Lerner, A. Bianchi, and A. Borman. *Proc. Soc. Exp. Biol. Med.*, 1960, **103**, 172.
[94] T. Takemoto, S. Arihara, Y. Hikino, and H. Hikino, *Tetrahedron Letters*, 1968, 375.
[95] T. Takemoto, Y. Hikino, T. Okuyama, S. Arihara, and H. Hikino, *Tetrahedron Letters*, 1968, 6095.
[96] S. Okui, T. Otaka, M. Uchiyama, T. Takemoto, H. Hikino, S. Ogawa, and N. Nishimoto, *Chem. Pharm. Bull. Japan*, 1968, **16**, 384.
[97] (*a*) P. Hocks, U. Kerb, R. Wiechert, A. Furlenmeier, and A. Fürst, *Tetrahedron Letters*, 1968, 4281; (*b*) H. Hikino, Y. Hikino, and T. Takemoto, *Tetrahedron Letters*, 1968, 4255, and references cited therein.
[98] (*a*) H. Gibian, International Symposium Drug Research, Montreal, June 12–14, 1967; (*b*) P. Hocks, A. Jeger, U. Kerb, R. Wiechert, A. Furlenmeier, A. Fürst, A. Langemann, and G. Waldvogel, *Angew. Chem. Internat. Edn.*, 1966, **5**, 673.
[99] M. N. Galbraith, D. H. S. Horn, E. J. Middleton, and R. J. Hackney, *Chem. Comm.*, 1968, 466.



(11)



(12)

group for sterically hindered hydroxy-substituents could be especially useful.

An active principle of two species of the *Meliaceae*, meliantriol (12), shows marked antifeeding activity against the desert locust, *Schistocerca gregaria*.[100] Because of its biological activity and the many structural features common to meliantriol and the MH-active steroids, an evaluation of its hormonomimetic behaviour would seem to be indicated.

It may be safely assumed that many more MH-like substances from both plant and animal kingdoms remain to be isolated and characterised. There is no reason to suppose that MH-active compounds must be steroidal in character. For example, not only do locust extracts containing 'ecdysone-λ' exert significant growth stimulatory effects in a dwarf pea gibberellin bioassay, but, reciprocally, the plant growth hormone gibberellic acid mimics the moulting effects of ecdysone when injected into locust larvae.[101] A more thorough investigation of the MH-effects of the gibberellins in particular, and plant hormones in general, is still to be reported.

5 Biogenesis

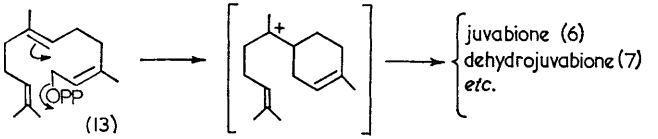
The predictably intriguing and definitive account of the biogenetic origins of the insect metamorphosis hormones is yet to be written.

Of the biogenesis of BH, essentially nothing is known.

From the C_{17} skeleton of JH, the structural resemblance of the hormone to the acyclic sesquiterpenoids, and the known presence of the latter in insects, an acyclic sesquiterpenoid (perhaps farnesol) could, by two C-methylations, account for the presence of JH. Alternatively, the two 'extra' C-atoms may originate earlier in the biogenetic scheme of things; homomevalonate has, for example, been implicated.[27] Conceivable, but less likely, is the possibility of four mevalonate units condensing with subsequent loss of a C_3-fragment. In *Samia cynthia* [2-^{14}C]mevalonate gives rise to labelled farnesol, farnesal, and nerolidol,[102] but incorporation of neither mevalonate nor methionine into JH has yet been demonstrated. The recent isolation[27] of a JH-active C_7-methyl analogue co-existing with the C_7-ethyl JH could have biogenetic relevance.

[100] D. Lavie, M. K. Jain, and S. R. Shpan-Gabrielith, *Chem. Comm.*, 1967, 910.
[101] D. B. Carlisle, D. J. Osborne, P. E. Ellis, and J. E. Moorhouse, *Nature*, 1963, **200**, 1230.
[102] P. Schmialek, *Z. Naturforsch.*, 1963, **18b**, 462.

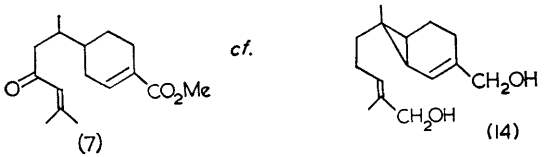
The biogenetic origin of juvabione and dehydrojuvabione, plant products with JH-like activity, might be easier to rationalise. The monocyclic sesquiterpenoidal character of these two compounds would logically suggest their farnesol origin,[103] perhaps by cyclisation of farnesylpyrophosphate (13):



(13)

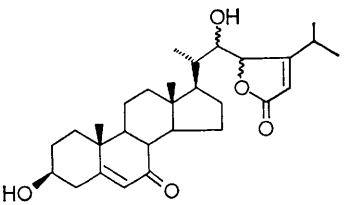
Implied is that dehydrojuvabione is a possible precursor of juvabione.

It is especially appealing to relate dehydrojuvabione (7) to sirenin, a plant sex hormone. Sirenin (14) is the sperm attractant of the water mould, *Allomyces*; its biogenetic origin has been attributed to cyclisation of *cis*-farnesylpyrophosphate.[104]



(7) (14)

The only other plant sex hormone of known structure is the steroid, antheridiol (15),[105] and it may similarly be compared to a number of previously described MH-active plant steroids.



(15)

From these comparisons two points emerge. Firstly, an examination of possible reciprocal biological activities is clearly indicated. Secondly, and contrary to earlier suggestions,[40,82] the plant may not elaborate molecules of the juvabione and MH-like steroid types primarily for defensive purposes; it could be that these or related compounds have a r le to play in the plant's reproductive processes, and that their defensive properties, if any, are coinci-

[103] W. Parker, J. S. Roberts, and R. Ramage, *Quart. Rev.*, 1967, **21**, 331.
[104] W. H. Nutting, H. Rapoport, and L. Machlis, *J. Amer. Chem. Soc.*, 1968, **90**, 6434.
[105] J. A. Edwards, J. S. Mills, J. Sundeen, and J. H. Fried, *J. Amer. Chem. Soc.*, 1969, **91**, 1248, and references cited therein.

dental or perhaps secondarily contrived. To this point is the observed[106] lack of moulting of the desert locust, *Schistocera gregaria*, when fed the bracken, *Pteridium aquilinum*, a plant known to contain both ecdysone and ecdysterone. Since bracken extracts are active by injection, it is Carlisle's conclusion that 'whatever function the ecdysones have in bracken and other plants, it is unlikely . . . that it is a form of defence against insects'. Support[107] and rejection[108] of this view have been expressed.

Although studies directed towards an understanding of the biogenesis of MH have been relatively neglected, some significant work has been carried out. By administering tritium-labelled cholesterol to *Calliphora* larvae and isolating radioactive ecdysone, Karlson[109] has been able to demonstrate that cholesterol is the precursor of ecdysone. This finding is consistent with the insect's dietary cholesterol requirement. Since the conversion of cholesterol to Δ^7-dehydrocholesterol has been established in aseptically reared insects,[110] dehydrogenation of cholesterol to the Δ^7-dehydro-derivative is probably the initial step in the biosynthesis of ecdysone. Extending the Karlson experiment,[109] Sauer *et al.*[111] showed that in seedling leaves of the conifer, *Podocarpus elata*, [4-^{14}C] cholesterol is converted to radioactive ecdysterone. The biogenesis of MH-active compounds found in insects may therefore be described, at least superficially, by the sequence:

cholesterol→7-dehydrocholesterol→ecdysone→ecdysterone.

A consideration of possible biogenetic pathways of the many plant steroids with MH-like activity would be a challenging exercise, but one which is considered to be beyond the scope of this Review.

6 Antagonists

An understanding of the chemistry and biochemistry of specific BH, JH, and MH antagonists could be of profound significance.

Gersch[11] regards the neurohormones C_2 (uric acid) and D_2 (xanthine) as antagonists to the neurohormones C_1 and D_1. More recent studies of Carlisle and Ellis[112] take advantage of the established correlation of secretory activity of the prothoracic gland with the diameter of the cell nuclei present. As in crustacea, where BH is opposed by a BH antagonist acting to prevent secretion of crustecdysone by the Y-organ, so Carlisle presents evidence that the lateral part of the protocerebrum of locusts produces an antagonist preventing secretion of 'hydroxylated ecdysone' by the prothoracic gland. The prothoracic gland, like the Y-organ, is thus homeostatically controlled by a delicate balance of antagonistic hormones which regulates the moulting process.

[106] D. B. Carlisle and P. E. Ellis, *Science*, 1968, **159**, 1472.
[107] T. Takemoto, S. Arihara, Y. Hikino, and H. Hikino, *Chem. Pharm. Bull. Japan*, 1968, **16**, 762.
[108] W. E. Robbins, J. N. Kaplanis, M. J. Thompson, T. J. Shortino, C. F. Cohen, and S. C. Joyner, *Science*, 1968, **161**, 1158.
[109] P. Karlson, and H. Hoffmeister, *Z. physiol. Chem.*, 1963, **331**, 298.
[110] W. E. Robbins, M. J. Thompson, J. N. Kaplanis, and T. J. Shortino, *Steroids*, 1964, **4**, 635.
[111] H. H. Sauer, R. D. Bennett, and E. Heftmann, *Phytochem.*, 1968, **7**, 2027.
[112] D. B. Carlisle and P. E. Ellis, *Nature*, 1968, **220**, 706.

Extirpation of the corpora allata reproduces all the manifestations of normal diapause (the state of physiological arrest). However, although surgically-induced diapause is completely reversed by implantation of corpora allata, normally diapausing beetles are unaffected by implantation of the glands. Bowers[113] concludes that there must therefore be, in normally diapausing beetles, a humoral JH antagonist, an antagonist, however, which is apparently not operative in allatectomy-induced diapause. Arguing that if diapause results from humoral inhibition of corpora allata secretion (when it should be possible to reverse diapause by supplying JH exogenously), Bowers demonstrated that his JH-active epoxyfarnesoate[34] indeed terminates diapause in the alfalfa weevil, *Hypera postica* (Gyllenhal).

In their in-depth exploration of the structural requirements of steroids needed to exert MH-like effects, the Prague group has described[114] a number of synthetic cholestanes, pregnanes, and androstanes which antagonise ecdysone. Inhibition of both postecdysial hardening and sclerotisation of the cuticle in *Pyrrhocoris apterus* is observed, the most active compounds[115] generally containing 6-keto- and 3β-hydroxy-functions. Most of the 6-ketosteroid derivatives tested on *Musca domestica* L. showed sterilising effects, but the structure–activity correlation, if any, is not obvious. The correlation may relate more to physico-chemical properties than to gross structural variations.

No published data yet indicate the presence of a naturally occurring ecdysone antagonist. Since MH is secreted by BH stimulation, however, it may be that the endogenous availability of a BH antagonist provides an adequate mechanism for exercising moulting control.

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[113] W. S. Bowers and C. C. Blickenstaff, *Science*, 1966, **154**, 1673.
[114] L. Lábler, K. Sláma, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1968, **33**, 2226.
[115] *Cf.* Syntex Corp., U.S.P. 3,365,474/1968.