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SYNTHESIS OF C_{27}-SUBSTITUTED ECDYSTEROIDS, POTENTIAL TOOLS FOR BIOCHEMICAL STUDIES

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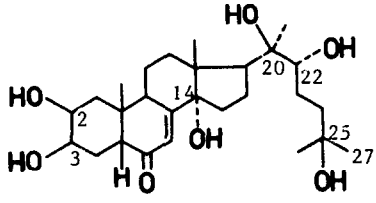
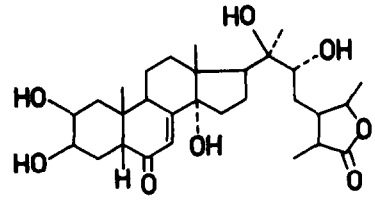
A sequence of selective protection procedures is described, facilitating the efficient cleavage of the ecdysteroid side-chain, which can then be reconstructed; in this way, C-27 ether-linked analogues of ecdysteroids have been synthesized, one of which displays highly significant biological activities.

One of us (BL) has participated for several years in a study of the biosynthesis and the regulation of action of the insect moulting hormones, the ecdysteroids (1). Extension of this work to the study of the receptors of these hormones would require the creation of suitable tools enabling one to use highly radioactive analogues, and/or to build affinity columns, etc. One approach appeared to be the synthesis of ecdysteroid analogues, carrying a stable substituent at a position leaving the biological activity unaltered: we describe in this Letter the



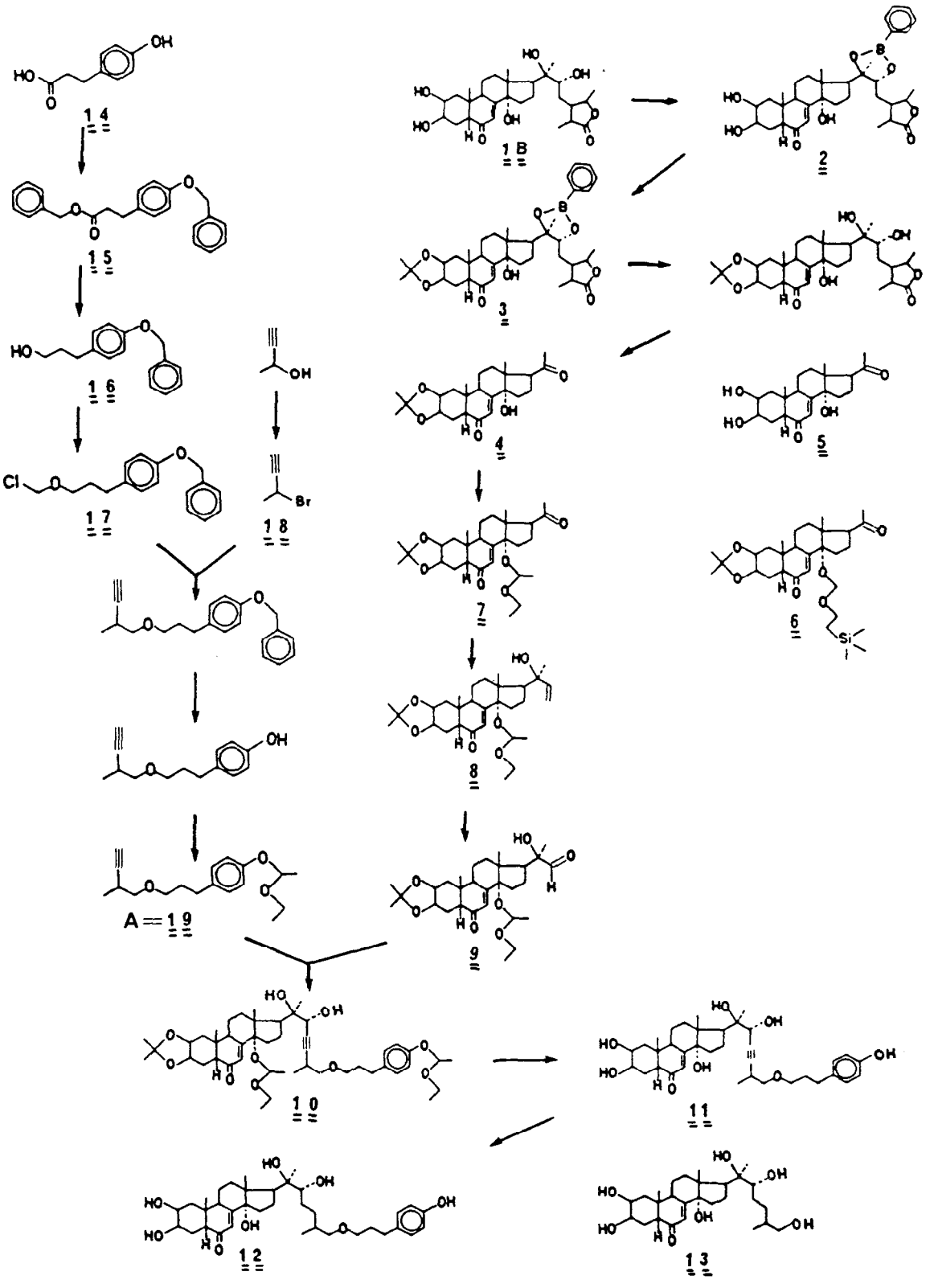
β-ecdysone
1A



cyasterone
1B

synthesis from 20,22-dihydroxy ecdysteroids, such as β-ecdysone 1A or cyasterone 1B, of one such analogue, 12, substituted at C-27 by an

To Harry Wasserman, whose permanent youth makes it a pleasure to age along with. G.O.


14
15
16
17
18
A = 19
18
2
3
4
5
6
7
8
9
10
11
12
13

ether-linked chain carrying a phenol group; this analogue displays, in three biological tests, an activity comparable to that of natural insect hormones.

The approach used was to cleave the side-chain of abundantly available phyto-ecdysteroids and to reconstruct it in a modified form. Cleavage was made possible by an efficient selective protection sequence for all the hydroxy groups of the starting molecule; reconstruction of the desired side-chain implied a hitherto little used condensation of chloroethers with acetylenic aluminium reagents, which proved very efficient. All the products have been fully characterised by their spectral data (MS, ^{1}H-NMR, and ^{13}C-NMR, the last ones being in particular very informative).

Selective protections: Selective cleavage of the 20,22-diol of β-ecdysone 1A or of cyasterone 1B was achieved by periodate oxidation of the 2,3-acetonide. Acetonide formation on the 2,3,20,22-tetrols is only marginally selective, in favour of the 20,22-acetonide (2). In contrast, phenylboronic acid (1.2 - 4 equ., r.t., THF or DMF, <30 min.) gives exclusively the 20,22-phenylboronates 2, stable enough to acid to be converted quantitatively into the corresponding 2,3-acetonides 3, by reaction with 2,2-dimethoxypropane and fused TsOH. Removal of the side-chain protection by neutral hydrogen peroxide is again nearly quantitative, and can be followed by periodate cleavage under phase-transfer conditions. Poststerone 2,3-acetonide 4 was thus obtained in up to 74% overall yield from 1A or 1B, and identified by hydrolysing it (Dowex 50WX4, MeOH, CH_2Cl_2, r.t., 3 d.) to free poststerone 5 (3) (direct comparison with an authentic sample by mp, mixed mp, MS and NMR).

The 14-hydroxyl group of 4 was next protected, either as the "SEM"-ether (2-trimethylsilyl-ethoxy)methyl-ether 6 (4), or as the acetal 7. Both have been fully processed, but the SEM-ether group was extremely resistant to hydrolysis, once the side-chain had been reconstructed. The route using the acetal 7 was therefore preferable, even though substances carrying this group are diastereomeric mixtures (no noticeable asymmetric induction, judging from their spectra), which have been purified only by "low resolution" chromatography, without recrystallisation.

The acetal 7 was converted by successive addition of vinylmagnesium bromide (THF, -50°; 20S 73%, 20R 8 %) and ozonation (CH_2Cl_2, -80°, 30-40 min. with tlc control, then Me_2S -80°, 0°, r.t.) into the hydroxyaldehyde 9, which was condensed with the magnesium derivative of the complex acetylenic synthon 19, the preparation of which is described below. The acetylenic bis-acetal acetonide 10 was fully deprotected by treatment with Dowex 50WX4, then hydrogenated on palladium (5) to the end-product 12 (25R/25S 1/1), a derivative of inokosterone 13, characterised by all its spectra. In Nature, inokosterone itself occurs as a 1/1 mixture of the C-25 diastereomers (5); we have therefore not attempted to separate the C-25 diastereomers of 12 in the final chromatographic purification of the final product by HPLC (C_{18} - inverse phase), but directly submitted to the bioassays.

Preparation of synthon A: Prévost and Gaudemar have described the condensation of aldehydes with allenic aluminium derivatives, themselves obtained from propargyl bromides; this gives mixtures of the homopropargylic and of the allenic alcohols (6) - other metals reportedly give exclusively the allenic derivatives . Gaudemar had replaced in these reactions the aldehyde by an α-chloroether, and had obtained a product shown by IR to be a 1/1 mixture of the homopropargylic and of the allenic ethers (7); however, L.Miginiac had obtained, from an α,β-dibromoether, exclusively the homopropargylic ether (8). Indeed, a wide variety of α - chloroethers, when worked up in the cold, have given us the corresponding homopropargylic ethers in yields of the order of 50%; we cannot exclude positively the formation of the allenic isomers, but these have not been detected, even on 200 MHz ^{1}H-NMR spectra.

Thus, for the preparation of synthon 19 = A, phloretic acid 14 was converted to the bis-benzylderivative 15 (NaH, $PhCH_2Br$, 0.1eq.$Bu_4N^+I^-$, THF/HMPA, refl., 15h, 98%),and reduced to the alcohol 16 (LAH, Et_2O, 95%). The crude chloromethylether 17 obtained with formaldehyde and hydrogen chloride was condensed in THF with the aluminium derivative obtained from the propargylic bromide 18. This was debenzylated, using Fujita's procedure (EtSH + BF_3.etherate) (9), and reprotected with ethylvinylether, to afford the acetylenic synthon 19 (1/1 mixture of diastereomers, not separated), the magnesium derivative of which was obtained by treatment with ethylmagnesium bromide, required to give 12.

The overall yield from cyasterone to the end-product 12 is 7.5 % for 10 steps.

Biological results: Three biological tests have been run to evaluate the activity of the end-product 12. The activity on the puffing of Drosophila salivary gland chromosomes was found to be markedly lower than that of ponasterone or β-ecdysone, and only slightly lower than that of ecdysone (e.g., same activity at 10^{-6} M as ecdysone at 10^{-7} M). On the test of evagination of imaginal disks, 12 was nearly as active as β-ecdysone, and it competed with β-ecdysone for its carrier protein at concentrations one order of magnitude higher.

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