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RUBROSTERONE, A METABOLITE OF INSECT METAMORPHOSING SUBSTANCE FROM *ACHYRANTHES RUBROFUSCA*: SYNTHESIS*

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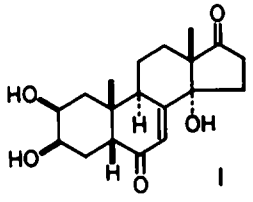
Abstract—Rubrosterone, a metabolite of ecdysterols isolated from *Achyranthes* spp. (Amaranthaceae), has been synthesized by two routes from ecdysterone.

RUBROSTERONE is an ecdysone analogue which was first isolated from *Achyranthes rubrofusca* Wight[1] and later from *A. fauriei* Léveillé et Vaniot and *A. obtusifolia Lamarck* (Amaranthaceae).[2] Earlier, on the basis of a chemical study,[3] stereostructure I was proposed for this natural product.



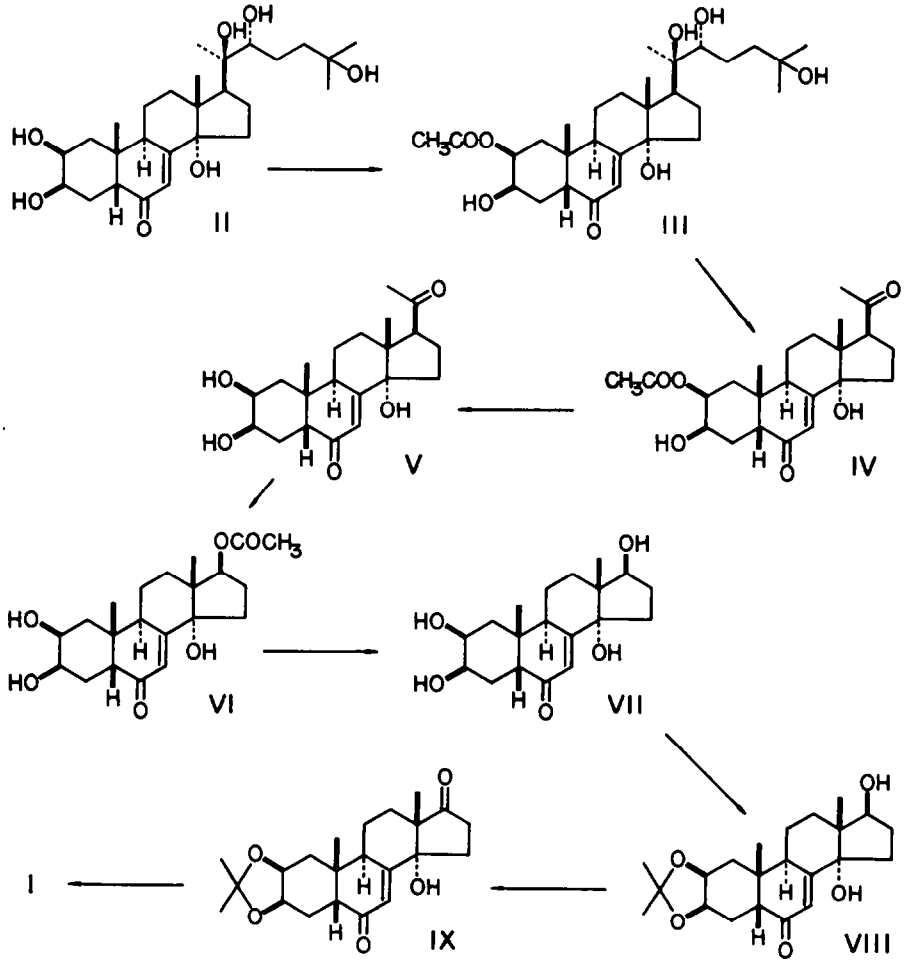
I

As rubrosterone together with ecdysterone and inokosterone occur in the same plants, rubrosterone is most probably a metabolite of the ecdysterols. Consequently, our interest was directed towards the biological activity of this metabolite. It has been found that rubrosterone has little effect upon induction of puparium formation of isolated abdomens of the flesh-fly (*Sarcophaga peregrina*); it exhibits no activity upon induction of imaginal development of dauer pupae (artificially made brainless pupae) of the silkworm (*Bombyx mori*),[3] but reveals high stimulating effect on protein synthesis in mouse liver.[4] Quite recently, it has been observed that rubrosterone, as with ecdysterone and inokosterone, promote the differentiation of cultured eye-antennal discs of the drosophila (*Drosophila melanogaster*) *in vitro*, and the activity is 10^6 times stronger than that of ecdysterone and inokosterone.[5] It is naturally expected that further interesting biological activities of this substance will be found. However, only very small amounts have hitherto been available for biological tests, since the contents of rubrosterone from plant sources is limited. Consequently, a synthetic method, making large amounts available for biological testing, is required.

* This paper is Part VI in the series on Steroids. Part V, T. Otaka, M. Uchiyama, T. Takemoto and H. Hikino, *Chem. Pharm. Bull.* (Tokyo) in press.

A successful synthesis of rubrosterone confirming the previous structural conclusion is described in this paper.*

The point of departure was the known ecdysterone (II) which had already been synthesized.[7] Ecdysterone (II) was converted *via* the 2-acetate (III) to the C_{21} methyl ketone acetate (IV) by periodate oxidation. Hydrolysis of the monoacetate (IV) with potassium hydrogen carbonate in aqueous methanol gave the known methyl ketone (V).[8] The synthesis of rubrosterone then became a matter of selective oxidation with peracid of the acetyl group of the dione (V). As the enone system in this nucleus is



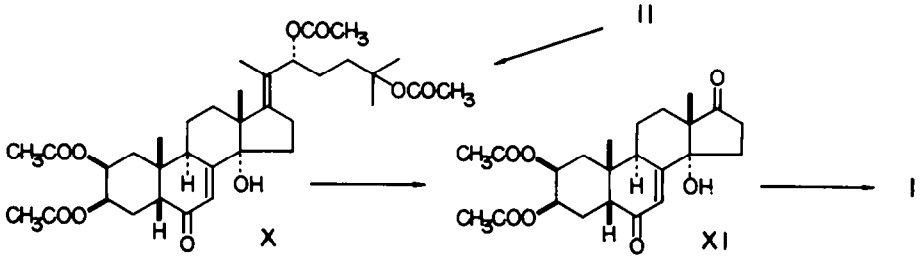
hindered, selective oxidation was accomplished simply by treatment of the methyl ketone (V) with an excess of pertrifluoroacetic acid. The UV (λ_{max} 242 mμ) and IR (ν_{max} 1727, 1242 and 1645 cm^{-1}) spectra of the product (VI) are consistent with the assigned structure and clearly exclude the alternative possibility that the enone group in the dione (V) was attacked by the reagent. On hydrolysis with potassium carbonate

* Part of the material here described was first presented in preliminary form (Ref. 6).

in aqueous methanol, the acetoxy-derivative (VI) yielded the tetra-ol (VII). In agreement with structure VII, it exhibits IR absorption at 3340 and 1648 cm^{-1}. At this stage, it was required that the 2β,3β-dihydroxy groups be protected from oxidation. This was conveniently achieved by conversion of the tetra-ol (VII) into the corresponding 2,3-acetonide (VIII) using acetone and a catalytic amount of *p*-toluenesulphonic acid. Oxidation of the alcohol (VIII) with chromium trioxide–pyridine complex afforded an oxidation product showing an IR max at 1731 cm^{-1} as expected for a ketonic CO function in a 5-membered ring, which was identified as rubrosterone acetonide (IX).[3] Hydrolysis of 2β,3β-acetonide in this system has hitherto been effected by an acidic reagent, e.g. 0·1N HCl in 10% aqueous THF.[9] However, since this nuclear structure is known to be labile under acidic conditions, it was desired that hydrolysis of the acetonide linkage is performed under milder, preferably neutral conditions. Previously, we found that heating of a 2β,3β-acetonide only with aqueous ethanol resulted in hydrolysis of the acetonide linkage to regenerate the free 2β,3β-diol.[10] The acetonide (IX) was then heated under reflux in aqueous ethanol leading to the expected removal of the protecting group to yield a hydrolysis product which was identified as the natural rubrosterone (I).

Thus a synthesis verifying both the structure and absolute configuration of rubrosterone has been completed. However, since the over-all yield of rubrosterone was very poor based on the amount of the starting ecdysterone (II), the route offers little promise of an adequate supply.

Meanwhile, it was found that acetylation of a certain ecdysterol under forced conditions led to the elimination of the C-20 OH group giving the C-17:C-20 double bond along with the esterification of the OH groups. Application of this method to ecdysterone (II) gave excellent results. Thus, heating with acetic anhydride in the presence of anhydrous sodium acetate at 140° for 30 min produced the anhydro-tetraacetate (X; 42% yield) together with the 2,3,22,25-tetraacetate (39% yield). Pro-



longation of the reaction resulted in the gradual formation of less polar products. However, when the reaction was carried out at 130°, genesis of the anhydro-derivative (X) was very slow. The yield of the anhydro-tetraacetate (X) varied but is satisfactory, when the yield of the by-product, the tetraacetate, which is convertible to the anhydro-derivative (X) on the same treatment, is taken into consideration. The NMR spectrum of the diene (X) is similar to that of ecdysterone tetraacetate. However, the C-21 Me signal at 1·23 ppm in the latter is shifted towards lowerfield appearing at 1·65 ppm in the former, showing that the reaction proceeded as expected. As has been mentioned, since the enone system in this nuclear structure is relatively inert, it was possible to cleave only the C-17:C-20 ethylenic linkage selectively. Thus, bubbling ozonized oxygen through a chloroform solution of the diene (X) gave an ozonolysis product

from which a main component was isolated (55% yield) and found identical with rubrosterone diacetate (XI).[3*] Hydrolysis of the diacetate (XI) with potassium carbonate in aqueous methanol furnished rubrosterone quantitatively. Thus, an alternative synthesis of rubrosterone by a short process consisting of three steps from the readily available ecdysterone (II), was completed in a much improved over-all yield.

The same method was also applied to inokosterone[11] to give a similar result.

The biological activities of the intermediates here prepared will be reported elsehere. Subsequent to our preliminary communication,[6] two alternative synthesis of rubrosterone were announced by two groups.[12, 13]

EXPERIMENTAL

M.ps are uncorrected. NMR spectra were determined on a Hitachi H-60 spectrometer unless specified to the contrary. The chemical shifts are given in ppm downfield from internal TMS and coupling constants (J) in Hz. Abbreviations: s = singlet, d = doublet, m = multiplet, dd = doublet of doublets, and br = broad peak.

Partial acetylation of ecdysterone with acetic anhydride in pyridine and chloroform. Ecdysterone (II; 500 mg) in Ac_2O (2 ml), pyridine (8 ml) and $CHCl_3$ (8 ml) was kept at 5° for 6 hr. After working up, the product (550 mg) was chromatographed over silica gel (13 g). Elution with $CHCl_3$–MeOH (30:1) and crystallization from MeOH–AcOEt gave III as colourless needles, m.p. 219–221°; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1740, 1241 (acetoxyl), 1637 (cyclohexenone); NMR ($CHCl_3$): 3H s at 0·85 ($C_{(18)}\underline{H_3}$), 3H s at 0·97 ($C_{(19)}\underline{H_3}$), 6H s at 1·24 ($C_{(26)}\underline{H_3}$, $C_{(27)}\underline{H_3}$), 3H s at 2·07 ($C\underline{H_3}$—COO—), 1H br at 3·06 ($C_{(9)}\underline{H}$), 1H dd at 3·42 ($C_{(22)}\underline{H}$), 1H br at 4·11 ($C_{(3)}\underline{H}$), 1H br at ~5·0 ($C_{(2)}\underline{H}$), 1H d at 5·82 (J = 2, $C_{(7)}\underline{H}$).

Periodate oxidation of ecdysterone 2-acetate. To the monoacetate (III; 400 mg) in MeOH (6 ml) was added $NaIO_4$ (200 mg) in water (6 ml) and the mixture was left standing at room temp for 1 hr. Isolation in the customary manner yeilded the product (340 mg) which on crystallization from MeOH afforded IV as colourless prisms, m.p.247–248°; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1737, 1241 (acetoxyl), 1695 (acetyl), 1640 (cyclohexenone); NMR ($CDCl_3$): 3H s at 0·63 ($C_{(18)}\underline{H_3}$), 3H s at 0·99 ($C_{(19)}\underline{H_3}$), two 3H s's at 2·09, 2·14 ($C\underline{H_3}$—COO—, $C_{(21)}\underline{H_3}$), 1H br at 4·10 ($C_{(3)}\underline{H}$), 1H br at 4·98 ($C_{(2)}\underline{H}$), 1H d at 5·84 (J = 2, $C_{(7)}\underline{H}$).

Hydrolysis of the methyl ketone acetate with alkali. Compound IV (180 mg) and $KHCO_3$ (100 mg) in aqueous MeOH (85%, 10 ml) were kept at room temp under N_2 for 2 hr. The product was isolated with n-BuOH and crystallized from MeOH–AcOEt to give V as colourless plates, m.p. ~245°; IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 1712 (acetyl), 1644 (cyclohexenone); NMR (C_5H_5N): 3H s at 0·68 ($C_{(18)}\underline{H_3}$), 3H s at 1·00 ($C_{(19)}\underline{H_3}$), 3H s at 2·12 ($C_{(21)}\underline{H_3}$), 1H d at 6·15 (J = 2, $C_{(7)}\underline{H}$).

Oxidation of the methyl ketone with pertrifluoroacetic acid. To H_2O_2 (90%, 0·4 ml) in CH_2Cl_2 (3 ml) $(CF_3CO)_2O$ (0·7 ml) was added dropwise with stirring at 0°. This mixture was added dropwise with stirring to V (160 mg) and ground Na_2HPO_4 (2 g) in CH_2Cl_2 (3 ml). The stirring was continued for 5 hr. The reaction mixture was diluted with water and extracted with n-BuOH. Working up in the usual way afforded the product (170 mg) which was crystallized from MeOH–AcOEt to give 17β-*acetoxy*-2β,3β,14α-*trihydroxy*-5β-*androst*-7-*en*-6-*one* (VI) as colourless prisms, m.p. 226–228°; UV λ_{max}^{KBr} mμ (log ε): 242 (4·10); IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1727, 1242 (acetoxyl), 1645 (cyclohexenone); NMR (C_5H_5N): 3H s at 0·85 ($C_{(18)}\underline{H_3}$), 3H s at 1·02 ($C_{(19)}\underline{H_3}$), 3H s at 2·06 ($C\underline{H_3}$—COO—), 1H d at 6·12 (J = 2, $C_{(7)}\underline{H}$).

Hydrolysis of the acetate with alkali. The acetate VI (83 mg) and K_2CO_3 (80 mg) in aqueous MeOH (80%, 4 ml) were set aside at room temp overnight. The mixture was diluted with water and extracted with n-BuOH. After working up, the product (76 mg) was crystallized from MeOH to yield 2β,3β,14α,17β-*tetrahydroxy*-5β-*androst*-7-*en*-6-*one* (VII) as colourless prisms, m.p. 268–270°; IR ν_{max}^{KBr} cm^{-1}: 3340 (OH), 1648 (cyclohexenone); NMR (C_5H_5N): 3H s at 0·97 ($C_{(18)}\underline{H_3}$), 3H s at 1·05 ($C_{(19)}\underline{H_3}$), 1H d at 6·15 (J = 2, $C_{(7)}$H).

* The diacetate (XI) has also been prepared from ponasterone A by Prof. K. Nakanishi, this University, using similar procedures: personal communication from Dr. Nakanishi.

Acetonide formation of the tetra-ol. The tetra-ol VI (25 mg) in anhyd acetone (5 ml) was stirred at room temp in the presence of *p*-toluenesulphonic acid (30 mg). After 20 min the mixture was diluted with water and extracted with AcOEt. After worked up, the product was crystallized from MeOH to give 2β,3β,14α,17β-*tetrahydroxy*-5β-*androst*-7-*en*-6-*one*-2,3-*acetonide* (VIII) as colourless needles, m.p. 246–248° (dec); IR ν_{max}^{KBr} cm^{-1}: 3340 (OH), 1645 (cyclohexenone).

Oxidation of the acetonide with chromium trioxide–pyridine complex. To the acetonide VIII (12 mg) in pyridine (0·6 ml), Cr_3O (22 mg) was added. After left standing overnight at room temp, the mixture was diluted with water, extracted with AcOEt, and worked up in the usual manner. The product on crystallization from MeOH gave IX as colourless prisms, m.p. 247–248·5°; IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1731 (cyclopentanone), 1677 (cyclohexenone). The identity with the acetonide prepared from the natural rubrosterone was confirmed by the usual criteria.

Hydrolysis of rubrosterone acetonide with aqueous ethanol. The acetonide IX (13 mg) in EtOH (1 ml) and water (1 ml) was heated under reflux for 24 hr. Evaporation of the solvent and crystallization from MeOH furnished I as colourless prisms, m.p. 246–248°; IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 1741 (cyclopentanone), 1644 (cyclohexenone). The identity with the natural rubrosterone was confirmed in the usual criteria.

Dehydration of ecdysterone with acetic anhydride in the presence of sodium acetate. Ecdysterone II (200 mg) in Ac_2O (2 ml) was kept at 140° in the presence of AcONa (300 mg) for 30 min. After isolation in the usual way, the product was chromatographed over silica gel (10 g).

Elution with benzene–AcOEt (8 : 1) gave 2β,3β,22(R),25-*tetraacetoxy*-5β-*cholesta*-7,17(20)-*dien*-6-*one* (X) as a colourless glass (110 mg); IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1738, 1248 (acetoxyl), 1661 (cyclohexenone); NMR ($CDCl_3$, 100 MHz): 3H s at 0·87 ($C_{(18)}\underline{H_3}$), 3H s at 1·03 ($C_{(19)}\underline{H_3}$), 6H s at 1·42 ($C_{(26)}\underline{H_3}$, $C_{(27)}\underline{H_3}$), 3H s at 1·65 ($C_{(21)}\underline{H_3}$), four 3H s's at 1·96, 1·99, 2·02, 2·10 ($C\underline{H_3}$—COO—), 1H br at 3·15 ($C_{(9)}\underline{H}$), 1H ddd at 5·08 (J = 4, 12, 3, $C_{(2)}\underline{H}$), 1H br at 5·34 ($C_{(3)}\underline{H}$), 1H br at 5·37 ($C_{(22)}\underline{H}$), 1H d at 5·91 ($C_{(7)}\underline{H}$).

Elution with AcOEt afforded ecdysterone tetraacetate (105 mg).

Ozonolysis of the anhydro-tetraacetate. The anhydro-tetraacetate X (110 mg) was dissolved in $CHCl_3$ (10 ml) and a stream of ozonized C_2 was passed through at 0° for 5 min. The mixture was diluted with AcOEt and hydrogenated over Pd–C (5%, 20 mg), the catalyst filtered off, and the solvent evaporated. The residue (101 mg) was chromatographed over silica gel (8 g). Benzene–AcOEt (5:1) eluate (41 mg) was crystallized from AcOEt–hexane to yield XI as colourless plates, m.p. 202–204°; IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1740 (cyclopentanone, acetoxyl), 1655 (cyclohexenone). Identification was carried out in the customary criteria.

Hydrolysis of rubrosterone diacetate with alkali. The diacetate XI (9 mg) and K_2CO_3 (9 mg) in aqueous MeOH (80%, 0·5 ml) were set aside at room temp for 30 min. TLC of the product showed that the reaction was complete. Isolation with n-BuOH in the customary manner gave the product (7 mg) which was crystallized from MeOH–AcOEt to furnish I as colourless prisms, m.p. 244–245°; IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 1740 (cyclopentanone), 1645 (cyclohexenone). Identification was performed in the usual criteria.

Dehydration of inokosterone with acetic anhydride in the presence of sodium acetate. Inokosterone (200 mg) in Ac_2O (2 ml) was kept at 140° in the presence of AcOEt (300 mg) for 30 min. Working up in the customary way yielded the product which was chromatographed over silica gel (10 g).

Elution with benzene–AcOEt (10 : 1) afforded 2β.3β,22(R(,26-*tetraacetoxy*-5β-*cholesta*-7,17(20)-*dien*-6-*one* as a colourless glass (124 mg); IR ν_{max}^{KBr} cm^{-1}: 3475 (OH), 1738, 1237 (acetoxyl), 1670 (cyclohexenone); NMR ($CHCl_3$): 3H s at 0·88 ($C_{(18)}\underline{H_3}$), 3H d at 0·95 (J = 6, $C_{(27)}\underline{H_3}$), 3H s at 1·03 ($C_{(19)}\underline{H_3}$), 3H s at 1·65 ($C_{(21)}\underline{H_3}$), four 3H s's at 1·99, 2·03, 2·03, 2·10 ($C\underline{H_3}$—COO—), 1H br at 3·15 ($C_{(9)}\underline{H}$). 2H m at 3·86 ($C_{(26)}\underline{H_2}$). 3H m at 5·0–5·4 ($C_{(22)}\underline{H}$, $C_{(2)}\underline{H}$, $C_{(3)}\underline{H}$). 1H d at 5·88 (J = 2, $C_{(7)}\underline{H}$).

Elution with AcOEt gave inokosterone 2,3,22,26-tetraacetate (59 mg).

Ozonolysis of the anhydro-tetraacetate. The anhydro-tetraacetate obtained (114 mg) was dissolved in $CHCl_3$ (10 ml) and ozonized at 0° for 5 min. After working up, the product (110 mg) was chromatographed over silica gel (8 g). Elution with benzene–AcOEt (5 : 1) and crystallization from AcOEt–hexane gave XI as colourless plates, m.p. 202–204°. The identity was confirmed in the usual criteria.

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