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STRUCTURE OF RUBROSTERONE, A NOVEL C_{19} METABOLITE OF INSECT-MOULTING SUBSTANCES FROM ACHYRANTHES RUBROFUSCA

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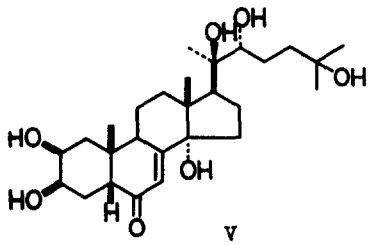
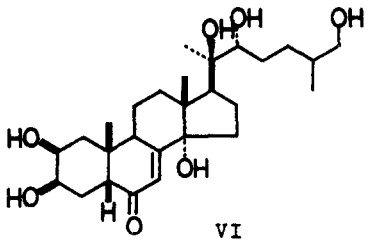
Recently a novel steroid, m.p. 230° (decomp.), along with ecdysterone (V) and inokosterone (VI), has been isolated from Achyranthes rubrofusca Wight (Amaranthaceae).[1,2] In the present



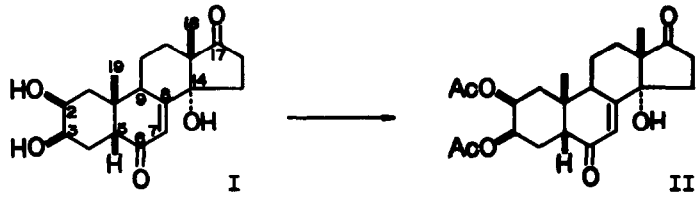
V



VI

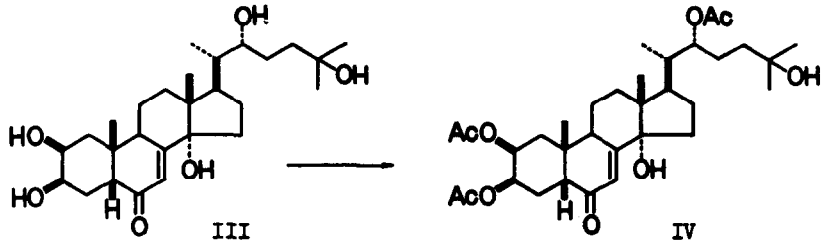
communication, we wish to report that the steroid now named rubrosterone is represented by stereoformula I and is consequently a possible metabolite of the insect-moulting substances in plants.

Rubrosterone, $C_{19}H_{26}O_5$,*1 MS: m/e 334 (molecular ion), $[\alpha]_D$ +119° (MeOH), gave a similar IR spectrum (KBr) to that of an insect-moulting substance, e.g., ecdysone (III),[3] a strong band at 3410 cm^{-1} (hydroxyl) and a characteristic band at 1646 cm^{-1} (cyclohexenone) being present. The most significant feature in the IR spectrum is a band at 1741 cm^{-1} (cyclopentanone). The NMR spectrum*2 shows two methyl singlets at 1.02 and 0.85 p.p.m. (the C-18 and C-19 protons, respectively). A UV maximum at 240 mμ and an NMR signal at 6.23 p.p.m. as well as the enone band in the IR spectrum demonstrate the presence of a β,β-disubstituted α,β-unsaturated ketone moiety. Since the CD curve of rubrosterone which reveals a positive Cotton effect ($[\theta]^{max}_{339}$ 32×10^2, dioxan) is similar to that of ecdysone (III)*3 ($[\theta]^{max}_{338}$ 42×10^2, dioxan), and rubrosterone, on acid treatment, gave a product which exhibited UV maxima at 295 and 241 mμ (the 7,14-dien-6-one and 8,14-



I II

dien-6-one chromophores, respectively)[3], the part-structure can be expanded to a 14-hydroxy-7-en-6-one system in the 5β-steroid nucleus. The presence of two secondary hydroxyls in rubrosterone was deduced by formation of the diacetate (II), $C_{23}H_{30}O_7$, m.p. 203–204°, IR (KBr): 3430 (hydroxyl), 1740, 1242 (acetoxyl), and 1659 cm^{-1} (cyclohexenone). The remaining hydroxyl group must be tertiary and as previously suggested should be located at C-14. The line positions and the splitting patterns of two carbinyl proton signals in the NMR spectrum of the diacetate (II) coincide with those of ecdysone triacetate (IV) (Table I), indicating that the two hydroxyls are situated at C-2 and 3, and are both β. This assignment was further supported by the chemical shift of the C-19 methyl protons of rubrosterone diacetate (II) in consistent with that of ecdysone tri-



III IV

TABLE I. Proton signals ($CDCl_3$, 100 MHz).[4]

	C-2α	C-3α	C-7	C-9	C-18	C-19	C-21	C-22	C-26	C-27
Ecdysone 2,3, 22-triacetate	5.05 ddd	5.34 ddd	5.87 d	3.11 ddd	0.67 s	1.02 s	0.94 d	4.9 ddd?	1.23 s	1.23 s
Rubrosterone 2,3-diacetate	4.94 ddd	5.32 ddd	5.94 d	3.11 ddd	0.85 s	1.04 s	--	--	--	--

TABLE II. Methyl chemical shifts (chloroform).

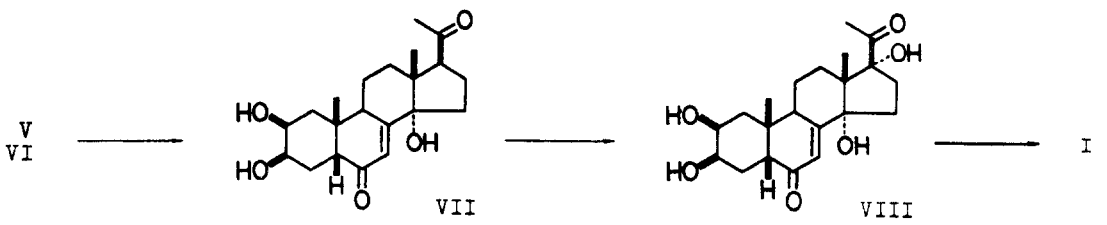
	Observed δ for ecdysone		Shift values[a] due to 17β-R[b]		Shift values[a] due to 17-oxo		Calculated δ for II		Observed δ for rubrosterone	
	C-19	C-18	C-19	C-18	C-19	C-18	C-19	C-18	C-19	C-18
Acetate	1.02	0.67	+0.01	+0.05	-0.02	-0.15	1.05	0.87	1.04	0.85

[a] Shift values were derived from the methyl signals of androstan-3α-ol, cholestan-3α-ol, and androsterone (p.p.m., plus sign represents an upfield shift).
[b] R=cholestane side-chain.

acetate (IV) (Table I and II). The presence of a single cis-1,2-glycol in rubrosterone was confirmed by periodate oxidation which resulted in the rapid consumption of 1 mole of the reagent. The saturated carbonyl group must be located at C-17, since it is in a five-membered ring and is not conjugated with the double bond at C-14:C-15 formed by elimination of the C-14 hydroxyl (vide supra). This together with the α-orientation of the C-14 hydroxyl group were supported by the CD curve of rubrosterone which exhibits a positive Cotton effect ($[\theta]_{286}^{max}$ 107×10^2, dioxan), and by the calculated chemical shift value for the C-18 methyl protons of a compound of structure II which is in agreement with the observed value for that of rubrosterone diacetate (Table II).

On the basis of the above observations we propose structure I for rubrosterone which, then, is the first substance possessing the etiocholane skeleton isolated from plant sources.*5

Rubrosterone is most probably biosynthesized from the insect-moulting steroids, ecdysterone (V) and inokosterone (VI), via the methyl ketone (VII) and the hydroxy-methyl ketone (VIII).



Horn et al. at first suggested that ecdysterone (crustecdysone) (V) was metabolized to the methyl ketone (VII);[4] but later concluded that this was unlikely since no methyl ketone (VII) could be detected in the crayfish extract,[5] from which ecdysterone was isolated.[4] However, the present isolation of rubrosterone, a possible metabolite of the insect-moulting substances, indicates the presence of a metabolic pathway, analogous to that from cholesterol to dehydroepiandrosterone, at least in the plant kingdom. It is very probable that the moulting hormones in animals are also metabolized through this pathway.

Thanks are due to Dr. M. C. Woods, Varian Associates, for the NMR spectra, and to Research Laboratories, Takeda Chemical Industries, Ltd., for the mass spectrum.

FOOTNOTES AND REFERENCES

*1 Satisfactory analytical figures were obtained for the compounds whose molecular formulae are shown.

*2 NMR spectra of rubrosterone and its diacetate were recorded on a Varian HA-100 spectrometer in

C_5D_5N and $CDCl_3$ solution, respectively. Chemical shifts (δ) are given in p.p.m. downfield from TMS as an internal reference. In the representation of data, s-singlet and d-doublet.

*3 Ecdysone was isolated from Lemmaphyllum microphyllum Presl (Polypodiaceae)[6] and Osmunda japonica Thunberg (Osmundaceae).[7]

*4 The methyl signal at 0.67 p.p.m. in ecdysone triacetate is shifted to 0.85 p.p.m. in ecdysterone triacetate, while that at 1.02 p.p.m. remains unchanged. Therefore, the signals at 0.67 and 1.02 p.p.m. in ecdysone triacetate must be attributed to the C-18 and 19 methyl protons, respectively. The previous assignment of the C-18 and 19 methyl signals in ecdysterone triacetate, cyasterone triacetate,[8] ecdysterone tetraacetate, and pterosterone tetraacetate[9] were reversed.

*5 A substance which has the androstane skeleton has already been reported to occur in a plant, Aplopappus heterophyllus Blake (Compositae).[10]

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