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STRUCTURE OF SENGOSTERONE, A NOVEL C_{29} INSECT-MOULTING SUBSTANCE FROM *CYATHULA CAPITATA*

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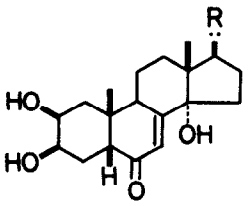
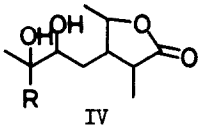
From the roots of *Cyathula capitata* Moquin-Tandon (Amaranthaceae), the C_{29} phytoecdysones, cyasterone (IV), capitasterone (V), amarasterone A (VI), and amarasterone B (VII) have hitherto been isolated.[1-3] Further survey has resulted in the isolation of another novel C_{29} analogue with insect moulting hormone activity for which the term sengosterone is proposed. In the present communication, we wish to provide evidence leading to the expression I for sengosterone which, therefore, may be the immediate metabolite of cyasterone (IV).

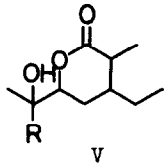
Sengosterone, $C_{29}H_{44}O_9$ (M-H_2O at m/e 518), m.p. 159~161°, $[\alpha]_D$ +39.6° (pyridine), shows positive color reactions for steroids. The IR spectrum, exhibiting a characteristic band at 1748 cm^{-1} (γ-lactone) as well as strong bands at 3425 (hydroxyls) and 1670 cm^{-1} (enone), is essentially identical with that of cyasterone (IV). The enone band along with a UV maximum at 240 mμ and an NMR signal (1H) at 6.22 p.p.m.* demonstrate the presence of the α,β-unsaturated ketone system as in cyasterone (IV). The NMR spectrum shows five methyl signals whose chemical shifts and splitting patterns are consistent with those of cyasterone (IV) with the exception that the signal corresponding to the C-19 methyl protons is shifted as compared with that of cyasterone (Table I). The above observations indicate that sengosterone may most probably be a monohydroxylated derivative of cyasterone (IV). In the mass spectrum of sengosterone, the peaks due to the side-chain fragments occur at m/e 201 (M-335), 183 (M-335-18), 157 (M-379), and 113 (M-423) which are also present in the spectrum of cyasterone (IV), showing that the side-chain contains no extra hydroxyl group. On the other hand, the peaks at m/e 379 (M-157), 361 (M-157-18), 343 (M-157-36), and 325 (M-157-54) attributed to the nucleus fragments are 16 mass units higher than the corresponding peaks in that of cyasterone (IV), a fact which indicates the extra hydroxyl to be located in the nucleus.

Acetylation of sengosterone gave the diacetate (II) and the triacetate (III). The IR spec-





IV



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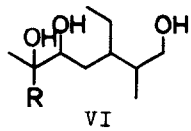

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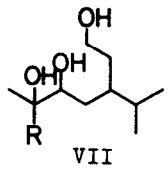


VII

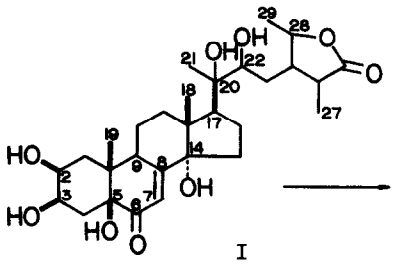
Table I. Methyl chemical shifts (pyridine).

		C-18	C-19	C-21	C-26	C-27	C-29
Ecdysterone	(IX)	1.19	1.06	1.55	1.34	1.34	--
Polypodine B	(VIII)[5]	1.19	1.10	1.55	1.35	1.35	--
Cyasterone	(IV)[1]	1.19	1.06	1.51	--	1.33	1.33
Sengosterone	(I)	1.21	1.13	1.56	--	1.36	1.34

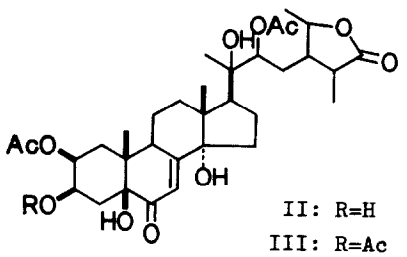
Table II. Proton signals ($CDCl_3$).

	C-2α	C-3α	C-7	C-9	C-18	C-19	C-21	C-22	C-27	C-28	C-29
Cyasterone 2,3,22-triacetate	~5.01 +	5.31 ddd	5.85 d	3.11 ddd	0.85 s	1.02 s	1.25 s	~4.98 +	1.28 d	4.10 dq	1.41 d
Sengosterone 2,3,22-triacetate	5.27 +	5.22 +	5.95 d	3.21 ddd	0.85 s	0.93 s	1.25 s	4.99 dd	1.28 d	4.11 dq	1.41 d
Sengosterone 2,22-diacetate	5.09 +	4.12 +	5.95 d	3.20 ddd	0.85 s	0.91 s	1.25 s	4.97 +	1.28 d	4.11 +	1.41 d

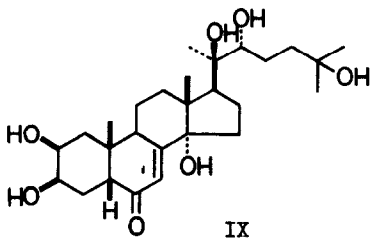
+ Patterns are unclear due to overlapping of the signals.



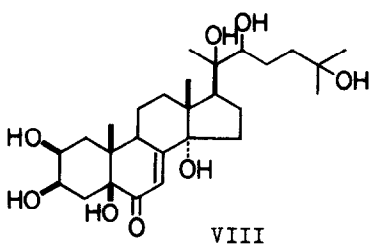
I



II: R=H
III: R=Ac



IX



VIII

trum of the triacetate (III) is again practically identical with that of cyasterone triacetate. Analysis of the NMR spectrum of the triacetate (III) (Table II) indicated that sengosterone has the same side-chain structure as cyasterone (IV). Since the NMR spectrum of the triacetate (III) exhibits no extra carbinyl hydrogen signal, the extra hydroxyl group must be tertiary and, consequently, situated at C-5, C-9, or C-17. Heating of sengosterone with hydrochloric acid in methanol afforded products showing UV maxima at 298 and 242 mμ (the 7,14-dien-6-one and 8,14-dien-6-one chromophores, respectively). If sengosterone had a 7-en-6-one-14,17-diol system, it would give, on acid treatment, a product which disclosed a maximum at a much longer wavelength due to a 7,14,16-trien-6-one chromophore, excluding the possibility that the extra hydroxyl is located at C-17. The presence of the C-9 hydrogen signals in the NMR spectra of sengosterone and its acetates (II and III) precluded the location at C-9. The situation of the extra hydroxyl at C-5 was finally secured as follows. In the NMR spectrum of sengosterone, there is no signal attributable to the 5β-hydrogen which appears at 2.94 p.p.m. in that of cyasterone (IV). The ORD and CD curves of sengosterone show a positive Cotton effect for the n-π^* transition of the carbonyl group (R-band). In comparison with the curves of cyasterone (IV), a hypsochromic shift of 10 mμ of the R-band, the disappearance of the fine structure of the band, and the increase of the amplitude (the molecular elipticity) are observed, demonstrating that the hydroxyl is situated α to the carbonyl and near the plane which compasses the carbonyl and its two adjacent carbon atoms (at C-5β).

In the NMR spectrum of the diacetate (II), an intramolecular nuclear Overhauser effect was observed between the hydrogen on the acetoxyl-bearing carbon (C-2) and the allylic hydrogen (C-9). Further, the signal shape of the hydrogen on the acetoxyl-bearing carbon (C-2) indicates it to be axially situated, while that of the hydrogen on the adjacent carbon attached to the hydroxyl (C-3) shows it to be equatorially oriented. Accumulated data establish that the C-2, C-3, and C-5 trihydroxyl groups are all in the β-orientation.

On the basis of the above evidence, sengosterone is concluded to be 5β-hydroxycyasterone (I).

Occurrence of an ecdysterol possessing a hydroxyl group at C-5 in a plant, Polypodium vulgare Linné (Polypodiaceae), has already been reported, i.e. polypodine B.[4] Although polypodine B is claimed to be 5β,20ξ-dihydroxyecdysone, no conclusive evidence for the configuration at C-2 and C-3 has been presented. Recently, isolation of a similar substance from the same plant source has been reported.[5] Although this substance was assigned to 5β-hydroxyecdysterone, it might possibly have the alternative 2α,3α,5α-trihydroxy-structure only based on the evidence provided. After the identity of polypodine B and 5β-ecdysterone has been shown,[6] the combined evi-

dence for both substances now demonstrates the 2β,3β,5β-trihydroxy-structure for polypodine B (VIII). This assignment is further supported by the close similarity of the chemical shift of the C-19 methyl signal in the NMR spectrum of polypodine B to that of sengosterone (Table I).

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FOOTNOTE AND REFERENCES

* The NMR spectra of the ecdysterol and its acetates are determined using a Varian HA-100 spectrometer in C_5D_5N and $CDCl_3$ solution, respectively. Chemical shifts are given in p.p.m. downfield from internal TMS. Abbreviations: s=singlet, d=doublet, q=quadruplet, and dd=doublet of doublets.

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6) K. Nakanishi, private communication.