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SENGOSTERONE, AN INSECT METAMORPHOSING SUBSTANCE FROM *CYATHULA CAPITATA*: STRUCTURE*

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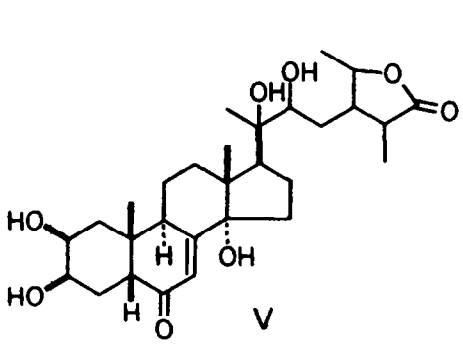
Abstract—A novel C_{29} insect metamorphosing substance, sengosterone, has been isolated from *Cyathula capitata* (Amaranthaceae) and shown to have structure I by chemical and physico-chemical studies.

IN RECENT years a number of ecdysterols, steroids possessing the insect-metamorphosing hormone activity, have been discovered from vegetable sources.

Inter alia, from the methanol extract of the crude drug Radix Cyathulae, the dried roots of *Cyathula capitata* Moquin–Tandon (Amaranthaceae), utilized as a tonic or a diuretic in Oriental medicine, we first isolated the unique C_{29} ecdysterol cyasterone[1] and later obtained the other C_{29} analogues capitasterone,[2] amarasterone A and amarasterone B,[3] and elucidated their structures as shown in formulas V, VI, VII and VIII, respectively.[1-3]



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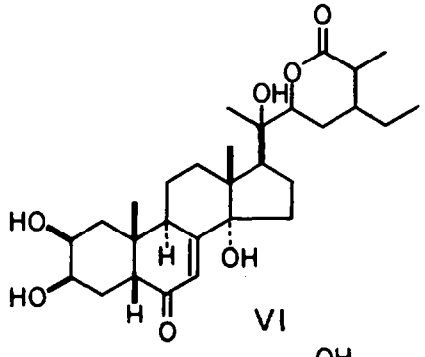

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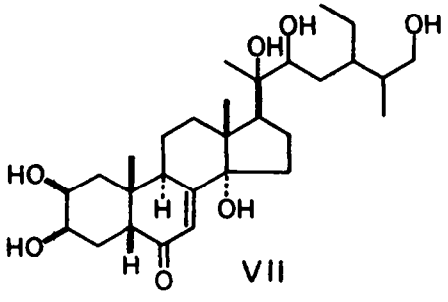

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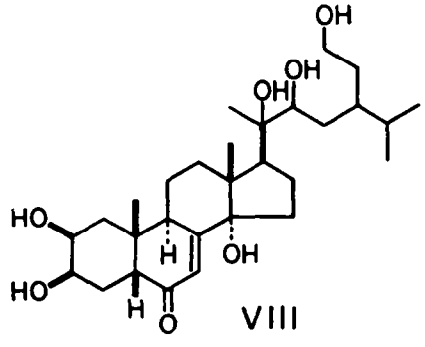
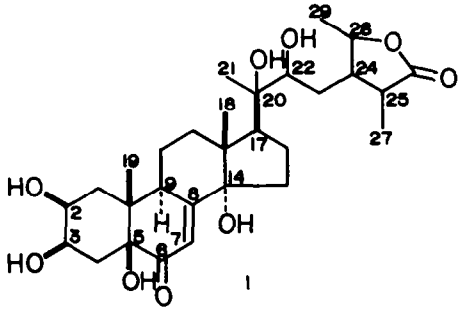


VIII

* This paper constitutes Part IX in the series on Steroids. Part VIII, H. Hikino, T. Kohama and T. Takemoto, *Phytochem.* in press.

Further survey on the extract by means of extensive chromatography has resulted in the isolation of another new C_{29} component showing the insect-metamorphosing hormone activity for which the term sengosterone is proposed.

The present paper reports the evidence which leads to the establishment of the structure along with the partial stereochemistry of sengosterone as depicted in formula I.*



I

Consideration of analytical values suggested the composition $C_{29}H_{44}O_9$ for sengosterone. This was strengthened by the mass spectrum which shows no molecular ion peak at *m/e* 536 but exhibits peaks at *m/e* 518 (4% relative to the base peak at *m/e* 43), 500 (10%), 482 (4%), 464 (2%) and 446 (1%) due to ions formed from the molecular ion by successive elimination of 1–5 molecules of water (Figs 1, 2).

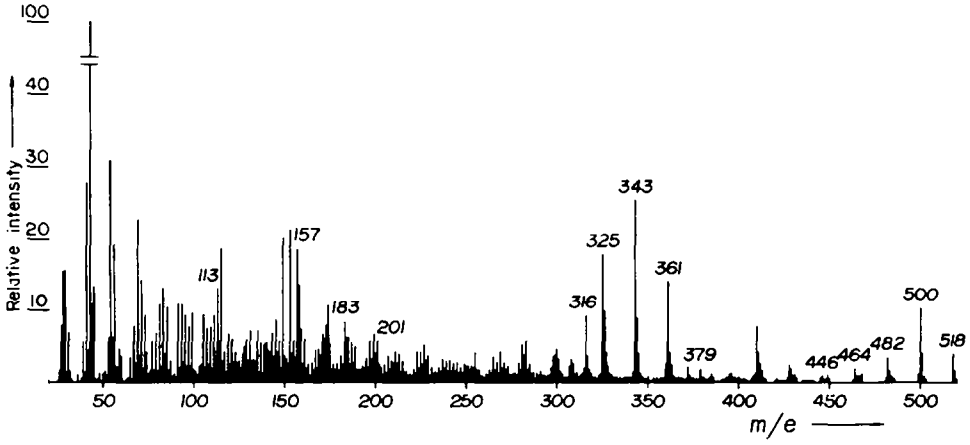


FIG. 1. Mass spectrum of sengosterone (70 eV).

* Part of the material reported herein formed the substance of a preliminary communication: *Tetrahedron Letters* 1417 (1969).

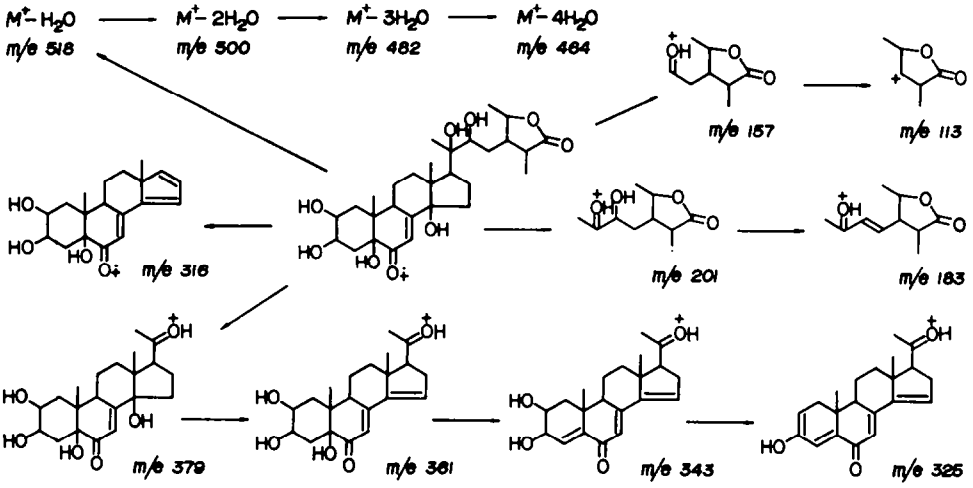


FIG. 2. Principal mass fragmentation of sengosterone.

The IR spectrum of sengosterone shows a characteristic band at 1748 cm^{-1} ascribable to a γ-lactone ring as well as strong bands at 3425 and 1670 cm^{-1} attributed to many OH's and an enone group, respectively. Therefore, sengosterone constitutes the second example of ecdysterols containing γ-lactone groupings, the first being cyasterone (V) isolated from the same source. Comparison of the spectrum with that of cyasterone was then carried out and, as a result, it was revealed that they were identical. The IR band at 1670 cm^{-1} together with the UV absorption at 241 nm and the NMR signal at 6·20 ppm demonstrates the presence of a β,β-disubstituted α,β-unsaturated ketone system as the 7-en-6-one chromophore in cyasterone. The NMR spectra of sengosterone and cyasterone in pyridine are also similar (Fig. 3, Table 1). Thus the presence of two tertiary Me's, one tertiary Me on an OH-bearing carbon, and two secondary Me's are indicated. Furthermore, the chemical shifts of these signals are compatible with those of cyasterone with the only exception that the signals assigned to the C-19 Me protons are separated by as much as 0·07 ppm. On the basis of the evidence already presented, our inference at this stage was that we were dealing with a substance closely related to cyasterone, which most probably is a monohydroxylated derivative of cyasterone or its isomer.

TABLE 1. METHYL CHEMICAL SHIFTS (PYRIDINE)

		C-18	C-19	C-21	C-26	C-27	C-29
Cyasterone	(V)[1]	1·19	1·06	1·51	—	1·33	1·33
Sengosterone	(I)	1·21	1·13	1·56	—	1·36	1·34
Ecdysterone	(IX)[9]	1·19	1·06	1·55	1·34	1·34	—
Polypodine B	(X)[5]	1·19	1·10	1·55	1·35	1·35	—
Pterosterone	(XI)[10]	1·18	1·05	1·54	1·00	1·00	—
Ponasterone C	(XII)[7]	1·17	1·12	1·54	1·00	1·00	—

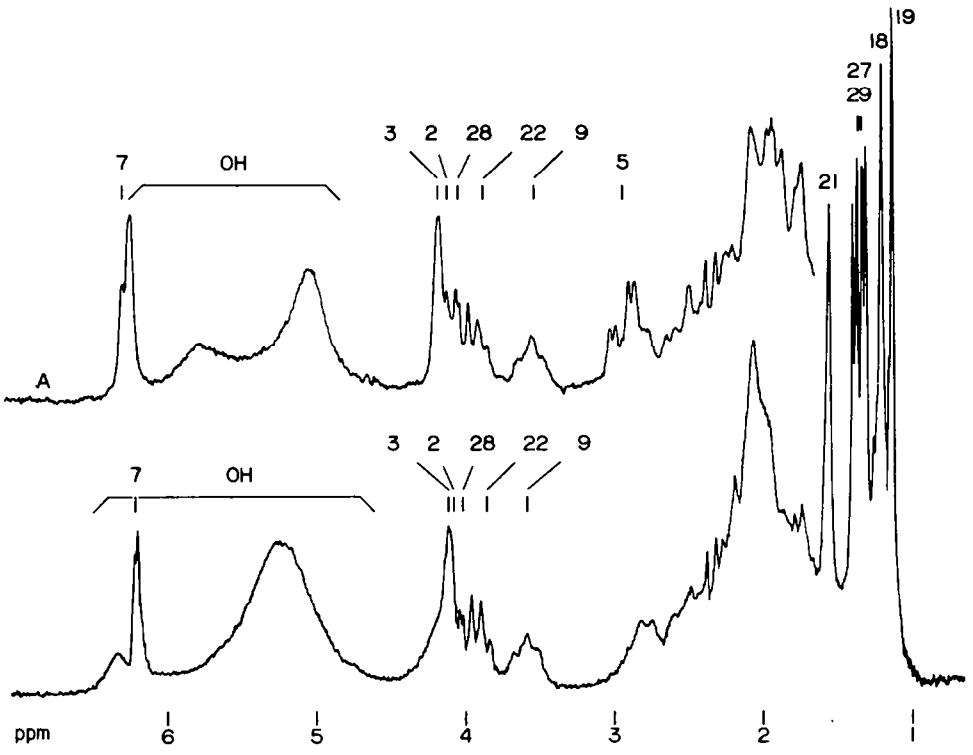
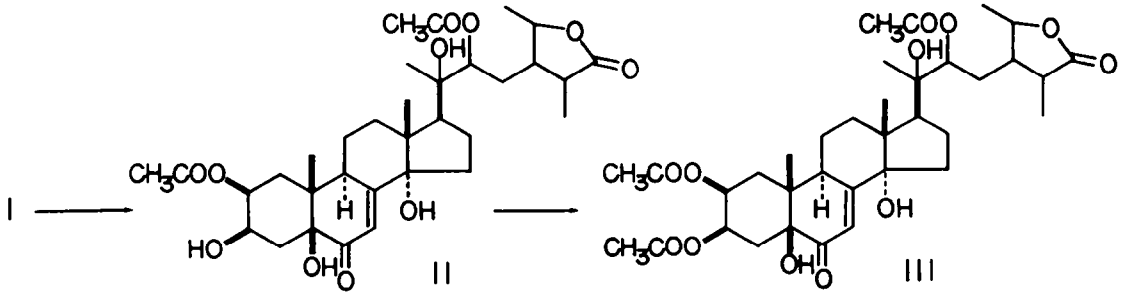


FIG. 3. NMR spectrum of sengosterone (C_5D_5N, 100 MHz). (A: that of cyasterone).

In order to confirm this assumption, sengosterone was acetylated with acetic anhydride in pyridine at room temperature overnight, conditions under which cyasterone forms a triacetate, to give two crystalline acetates, the diacetate (II) and the triacetate (III). The IR spectrum of the triacetate (III) is again practically identical



with that of cyasterone triacetate. The NMR spectrum of the triacetate (III) is also similar to that of cyasterone triacetate (Fig. 4, Table 2). Thus the chemical shifts and coupling patterns of certain signals coincide remarkably with those of the signals for the hydrogens on the carbons involved in the side-chain of cyasterone triacetate.

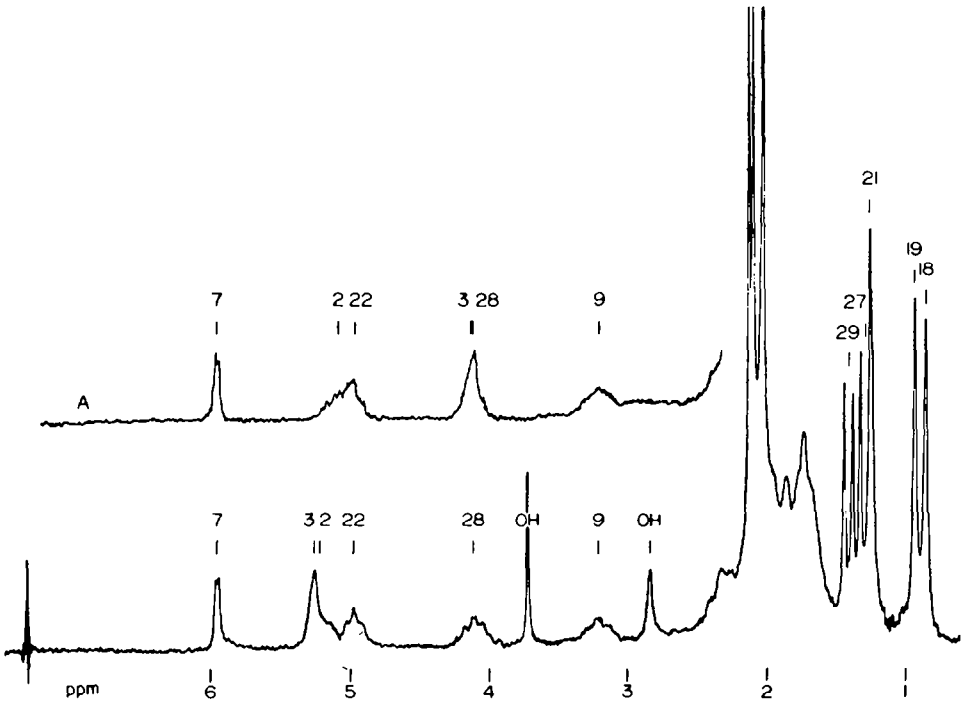


FIG. 4. NMR spectrum of sengosterone triacetate ($CDCl_3$, 100 MHz).
(*A*: that of sengosterone diacetate).

Furthermore, the signals were analyzed with the aid of double resonance experiments, the same side-chain structure for sengosterone as that of cyasterone being established.

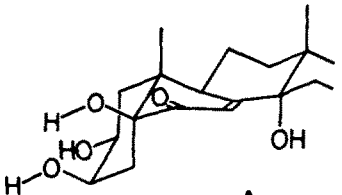
As suggested previously, sengosterone has an extra OH group as compared with cyasterone. The tertiary nature of the OH was demonstrated by the facts that sengosterone, as cyasterone, gives a triacetate and that the NMR spectrum of the triacetate (III) shows no extra carbinyl H signal other than the signals present in that of cyasterone triacetate (Fig. 4). However, as described above, the NMR spectrum of the triacetate (III) precluded the possibility that the OH is attached to any position of the side-chain. This assignment was fully supported by the mass spectral data (Figs 1, 2). Thus, the characteristic peaks appear at *m/e* 201 (M-335, 6%), 183 (M-335-18, 8%), 157 (M-379, 19%) and 113 (M-423, 13%) due to the side-chain fragments which are also present in the spectrum of cyasterone,[1] demonstrating that the side-chain structure contains no extra OH group. On the other hand, the prominent peaks at *m/e* 379 (M-157, 2%), 361 (M-157-18, 14%), 343 (M-157-36, 25%) and 325 (M-157-54, 18%) attributed to the nucleus fragments are 16 mass units higher than the corresponding peaks in that of cyasterone. Further, the nucleus fragment formed by the complete loss of the side-chain at C-17 and a subsequent loss of one molecule of water and a hydrogen occurs at *m/e* 316 which is also 16 mass units higher than the corresponding peak in cyasterone. These observations confirm the additional OH to be located in

TABLE 2. PROTON SIGNALS ($CDCl_3$)

	C-2α	C-3α	C-7	C-9	C-18	C-19	C-21	C-22	C-26	C-27	C-28	C-29
Cyasterone	~5·01	5·31	5·85	3·11	0·85	1·02	1·25	~4·98	—	1·28	4·10	1·41
2,3,22-triacetate[1]	+	ddd	d	ddd	s	s	s	+		d	dq	d
Sengosterone	5·27	5·22	5·95	3·21	0·85	0·93	1·25	4·99	—	1·28	4·11	1·41
2,3,22-triacetate	+	+	d	ddd	s	s	s	dd		d	dq	d
Sengosterone	5·09	4·12	5·95	3·20	0·85	0·91	1·25	4·97	—	1·28	4·11	1·41
2,22-diacetate	+	+	d	ddd	s	s	s	+		d	+	d

† Patterns are unclear due to overlapping of the signals.

the nucleus. Therefore, only three positions C-5, C-9 and C-17 remained for the location of the extra OH group. Heating of sengosterone in methanol in the presence of hydrochloric acid was performed to give products possessing UV maxima at 298 and 242 nm as with cyasterone.[1] This change cannot be rationalized in terms of the 7-en-6-one-14,17-diol system for sengosterone, which is expected to give a maximum at a much longer wavelength due to the 7,14,16-trien-6-one chromophore, excluding the possibility that the extra OH is situated at C-17. The presence of the C-9 H signals in the NMR spectra of sengosterone and its acetates (II and III) precludes the location at C-9 (Figs 3, 4). The situation of the extra OH at C-5 thus deduced was finally secured by the following facts. (1) Although the spectra were measured in KBr disks, the IR band attributed to the C=O stretching vibration of sengosterone appears at 1670 cm^{-1} which is displaced by 20 cm^{-1} towards a shorter wavelength as compared with that of cyasterone, indicating that an oxygen function is situated α to the CO group and near the plane which compasses the CO and its two adjacent C atoms as shown in perspective A. (2) The ORD and CD curves of sengosterone show positive Cotton effects for the n-π^* transition of the CO group (R-band) as with cyasterone. However,



A

comparison of the ORD and CD curves of sengosterone with those of cyasterone reveals that a hypsochromic shift of 10 nm of the R-band, the disappearance of the fine structure of the band, and the increase of the amplitude (the molecular ellipticity) are observed (Figs 5, 6, Table 3), demonstrating that the 5β-hydrogen in cyasterone is replaced by a 5β-OH group in sengosterone. (3) In the NMR spectrum of sengosterone, there is no signal attributable to the 5β-hydrogen which occurs at 2·94 ppm as a doublet of doublets in the spectrum of cyasterone (Fig. 3).[1]

Since the gross structure has thus been settled, the remaining problem is now the stereochemistry. As described above, physico-chemical properties of sengosterone indicate that its stereochemistry is the same as that of cyasterone but except that the configurations of the C-2 and C-3 substituents remain indefinite.

In the NMR spectrum of the diacetate (II), an intramolecular nuclear Overhauser effect (ca. 7%) was observed between the hydrogen on the acetoxyl-bearing carbon (C-2) and the allylic hydrogen (C-9), indicating both the hydrogens to be located in the spatially close relationship. Further, the signal shape of the hydrogen on the acetoxyl-bearing carbon (C-2) shows it to be axially situated, while that of the hydrogen on the adjacent carbon attached to the OH (C-3) indicates it to be equatorially oriented (Fig. 4), a fact which demonstrates that the C-2 and C-3 dihydroxyl groups are both in the β-configuration. This was further corroborated by the formation of the

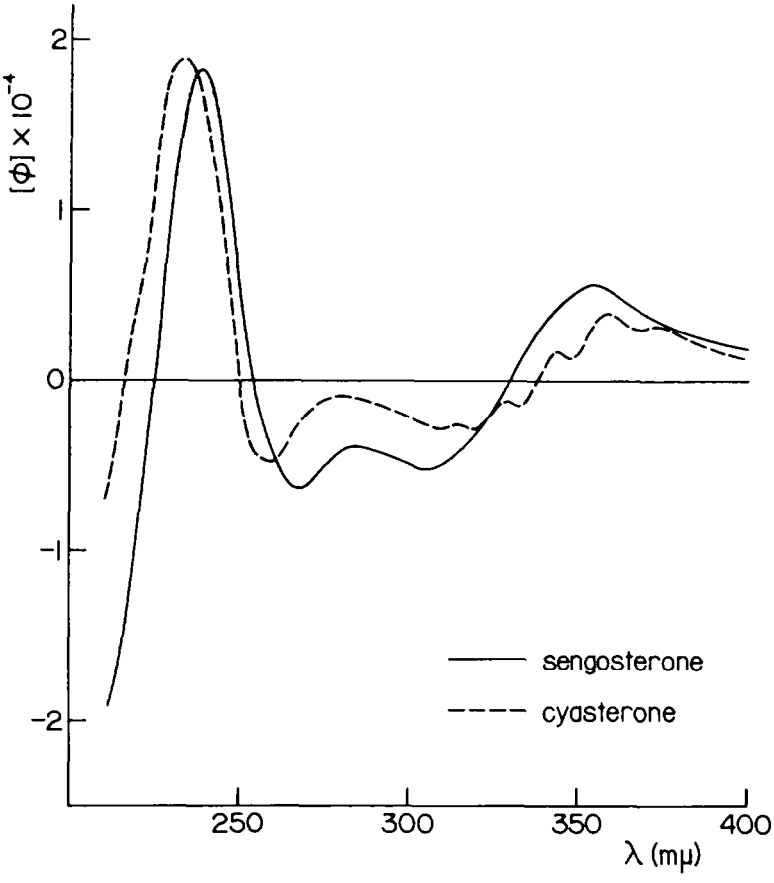


FIG. 5. ORD curves of sengosterone and cyasterone (dioxan).

TABLE 3. COTTON EFFECTS FOR n-π^* TRANSITION

		Maximum (nm)	Molecular elipticity ([θ])	Aplitude (a)
Cyasterone	(V)	338	+5100	+68
Sengosterone	(I)	328	+7490	+107
Ecdysterone	(IX)	339	+5280	+72
Polypodine B	(X)[4]	329·5	+9600	—
Pterosterone	(XI)[10]	339	+4210	+69
Ponasterone C	(XII)[7, 8]	324	+9900	+110

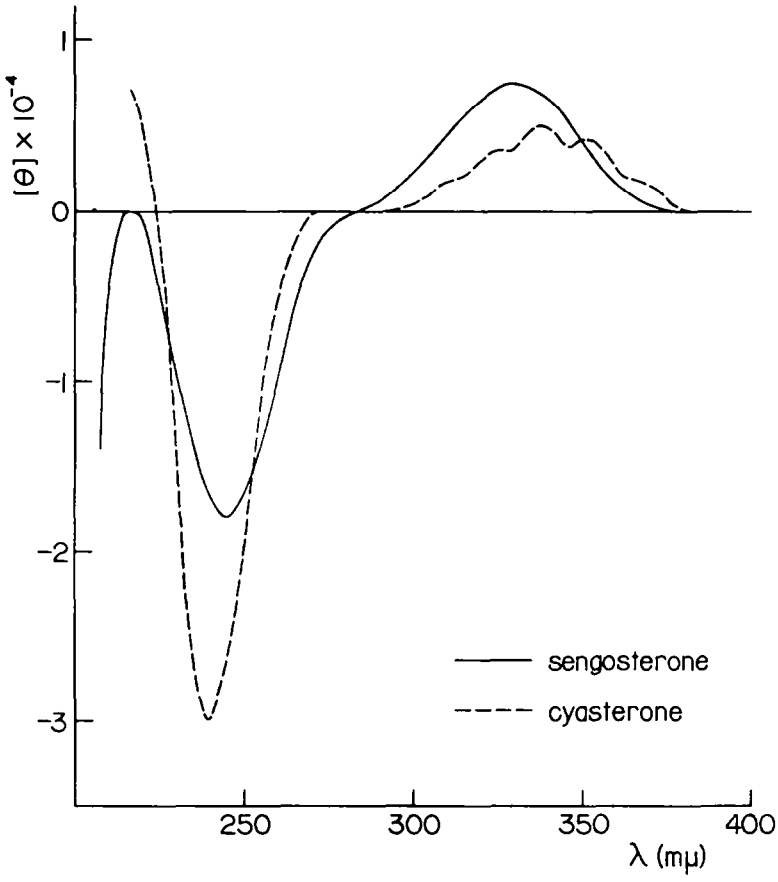
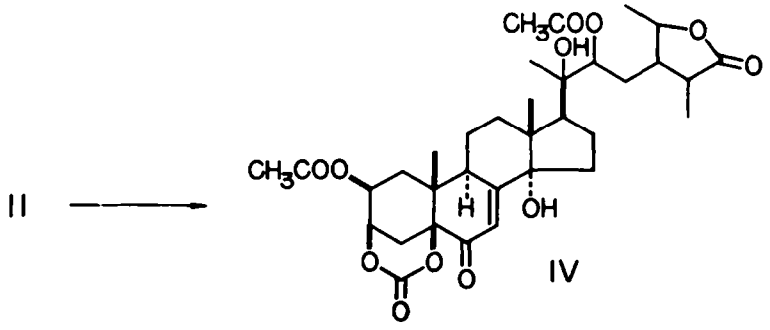


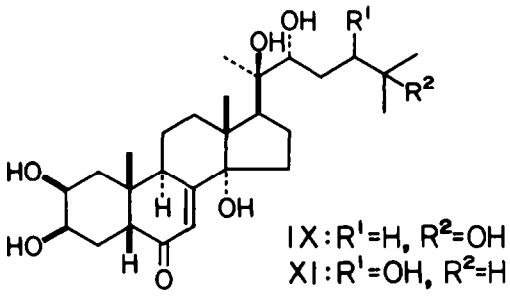
FIG. 6. CD curves of sengosterone and cyasterone (dioxan).

3,5-carbonate (IV) from the diacetate (II), the *cis* relationship of the C-3 and C-5 dihydroxyl groups being established.

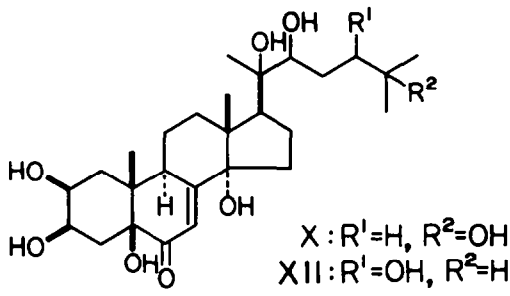
On the basis of the above evidence, it is concluded that sengosterone is 5β-hydroxycyasterone (I) and may be the immediate metabolite of cyasterone. The stereochemistry of the side-chain of sengosterone, as with that of cyasterone remains to be determined.



Occurrence of an ecdysterol possessing an OH 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 configurations at C-2 and C-3 has been presented. Later, isolation of a similar substance from the same 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-hydroxy-ecdysterone has been shown,[6] the combined evidence for both substances now demonstrates the 2β, 3β, 5β-trihydroxy-structure for polypodine B (X). Ponasterone C, a minor phytoecdysone from *Podocarpus nakaii* Hayata



IX : R¹=H, R²=OH
XI : R¹=OH, R²=H



X : R¹=H, R²=OH
XII : R¹=OH, R²=H

(Podocarpaceae), was first considered to be 2α,3α,14α,20ξ,22ξ,24ξ-hexahydroxy-5β-cholest-7-en-6-one[7] but recently revised to have the structure XII also possessing the 5β-OH group.[8]

As mentioned above, in the NMR spectra, the C-19 Me protons in sengosterone resonate apart by 0·07 ppm from those in cyasterone, the difference being also found in the ecdysterone and pterosterone series (Table 1). The changes in the ORD and CD curves caused by the replacement of 5β-hydrogens by 5β-OH groups, described above, are observed also in the other series (Table 3).

It is worthy to note the silica gel TLC of sengosterone and cyasterone. Thus, since sengosterone is a hydroxylated derivative of cyasterone, the former expectedly has a smaller mobility than the latter, when developed with a mixture of ethyl acetate and methanol. However, the R_f values of both substances are reversed using a mixture of chloroform and methanol as developing solvent (Table 4). Similar inversions are also observed in the other pairs of ecdysterols, *viz.*, ecdysterone (IX) and polypodine B (X), and pterosterone (XI) and ponasterone C (XII) (Table 4).

TABLE 4. R_f VALUES ON SILICA GEL THIN LAYER CHROMATOGRAPHY

		Developing solvents	
		AcOEt-MeOH (10:1)	$CHCl_3$-MeOH (5:1)
Cyasterone	(V)	0·19	0·27
Sengosterone	(I)	0·15	0·31
Ecdysterone	(IX)	0·13	0·16
Polypodine B	(X)	0·09	0·19
Pterosterone	(XI)	0·30	0·28
Ponasterone C	(XII)	0·25	0·32

EXPERIMENTAL

M.ps are uncorrected. NMR spectra were determined on a Varian HA-100 spectrometer. Chemical shifts are expressed in ppm downfield from internal TMS and coupling constants in Hz. Abbreviations: s = singlet, d = doublet, m = multiplet, dd = doublet of doublets and br = broad.

Isolation of sengosterone. The crude drug Radix Cyathulae (19 kg), the dried roots of *Cyathula capitata* Moquin–Tandon (Amaranthaceae), was extracted 5 times with refluxing MeOH (25 L each) for 7 hr (each extraction). The combined MeOH soln was concentrated to yield an extract (7·6 kg), which on extraction with AcOEt and evaporation gave a residue (170 g). Chromatography of the residue (170 g) over alumina (750 g), elution with AcOEt and crystallization from MeOH furnished V as colorless needles (4·0 g). The mother liquor was evaporated and chromatographed repeatedly over silica gel to give I as a colorless amorphous mass (50 mg), $[\alpha]_D$ +39·6° (*c* 0·69, C_5H_5N), ORD (*c* 0·2039, dioxan): $[\phi]_{400}$ +1960, $[\phi]_{356}$ +5620, $[\phi]_{304}$ −5050, $[\phi]_{283}$ −3720, $[\phi]_{267}$ −6320, $[\phi]_{238}$ +18420, $[\phi]_{212}$ −18850, CD (*c* 0·2039, dioxan): $[\theta]_{380}$ 0, $[\theta]_{328}$ +7490, $[\theta]_{283}$ 0, $[\theta]_{245}$ −18070, $[\theta]_{220-214}$ 0, $[\theta]_{206}$ −30660; UV λ_{max}^{MeOH} nm (log ε): 241 (4·01); IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 1748 (γ-lactone), 1670 (cyclohexenone); NMR (C_5D_5N): 3H s at 1·13 ($C_{(19)}\underline{H}_3$), 3H s at 1·21 ($C_{(18)}\underline{H}_3$), 3H d at 1·34 (J = 6, $C_{(29)}\underline{H}_3$), 3H d at 1·36 (J = 7, $C_{(27)}\underline{H}_3$), 3H s at 1·56 ($C_{(21)}\underline{H}_3$), 1H ddd at 3·58 ($C_{(9)}\underline{H}$), 3H m at 3·8–4·1 ($C_{(22)}\underline{H}$, $C_{(28)}\underline{H}$, $C_{(2)}\underline{H}$), 1H single peak at 4·10 ($C_{(3)}\underline{H}$), 1H d at 6·20 (J = 2, $C_{(7)}\underline{H}$). Liebermann–Burchard reaction: positive (red).

Acetylation of sengosterone. Sengosterone (200 mg) in Ac_2O (1 ml) and pyridine (2 ml) was left standing at room temp overnight. Upon isolation in the usual manner, the product (210 mg) was chromatographed over silica gel (30 g).

Elution with benzene-AcOEt (10:7) and crystallization from MeOH gave III as colorless plates (100 mg), m.p. 224–225°; IR ν_{max}^{KBr} cm^{-1}: 3540, 3460 (OH), 1776 (γ-lactone), 1738, 1235 (acetoxyl), 1675 (cyclohexenone); NMR ($CDCl_3$): 3H s at 0·85 ($C_{(18)}\underline{H}_3$), 3H s at 0·93 ($C_{(19)}\underline{H}_3$), 3H s at 1·25 ($C_{(21)}\underline{H}_3$), 3H d at 1·28 (J = 7, $C_{(27)}\underline{H}_3$), 3H d at 1·41 (J = 6, $C_{(29)}\underline{H}_3$), three 3H s's at 2·02, 2·09, 2·12 ($C\underline{H}_3$—COO—), 1H ddd at 3·21 (J = 2·8, 10, $C_{(9)}\underline{H}$), 1H dq at 4·11 (J = 7, 6, $C_{(28)}\underline{H}$), 1H dd at 4·99 ($C_{(22)}\underline{H}$), 1H br peak at 5·22 ($C_{(3)}\underline{H}$), 1H single peak at 5·27 ($C_{(2)}\underline{H}$), 1H d at 5·95 (J = 2, $C_{(7)}\underline{H}$).

Further elution with benzene-AcOEt (1:2) and crystallization from MeOH yielded II as colorless needles (60 mg), m.p. 248–250°; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1778 (γ-lactone), 1740, 1230 (acetoxyl), 1675 (cyclohexenone); NMR ($CDCl_3$): 3H s at 0·85 ($C_{(18)}\underline{H}_3$), 3H s at 0·91 ($C_{(19)}\underline{H}_3$), 3H s at 1·25 ($C_{(21)}\underline{H}_3$), 3H d at 1·28 (J = 7, $C_{(27)}\underline{H}_3$), 3H d at 1·41 (J = 6, $C_{(29)}\underline{H}_3$), two 3H s's at 2·00, 2·01 ($C\underline{H}_3$—COO—), 1H ddd at 3·20 (J = 2, 8, 10, $C_{(9)}\underline{H}$), 1H br peak at 4·10 ($C_{(28)}\underline{H}$), 1H single peak at 4·12 ($C_{(2)}\underline{H}$), 1H br peak at 4·99 ($C_{(22)}\underline{H}$), 1H br peak at 5·09 ($C_{(3)}\underline{H}$), 1H d at 5·97 (J = 2, $C_{(7)}\underline{H}$).

Acid treatment of sengosterone. Sengosterone (0·12 mg) in dil HCl (conc HCl-MeOH = 1:100, 4 ml) was heated under reflux for 10 min to afford the mixture of 8,14-dien-6-one and the 7,14-dien-6-one, UV $\lambda_{max}^{MeOH(HCl)}$ nm: 242, 298.

Cyclic carbonate formation of sengosterone 2,22-diacetate. To the diacetate (II; 20 mg) in pyridine (1 ml) was added $COCl_2$ in toluene (30%, 1 ml) at 0°. After the reaction temp was gradually raised to room temp, water was added. Extraction with AcOEt gave an oil (15 mg) which was subjected to preparative TLC on silica gel plates developed with AcOEt. The zones having R_f ca. 0·83 were removed and extracted with AcOEt to afford an oil (6 mg) which on crystallization from AcOEt gave IV as colorless needles (4 mg), m.p. 230–232°, IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1775, 1765 (γ-lactone, carbonate), 1740, 1220 (acetoxyl), 1680 (cyclohexenone); MS *m/e* (relative intensity (%)): 584 (0·2), 542 (0·5), 524 (1), 506 (2), 464 (2), 447 (2), 446 (3), 403 (1), 385 (1), 343 (1), 325 (5), 243 (3), 183 (11), 199 (6), 113 (11), 28 (100).

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