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CYASTERONE, AN INSECT METAMORPHOSING SUBSTANCE FROM *CYATHULA CAPITATA*: ABSOLUTE CONFIGURATION*

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Abstract—Chemical and physico-chemical studies on cyasterone and its derivatives (IV-VII) have established the stereostructure of cyasterone as shown in formula II.

CYASTERONE, a constituent of the crude drug Radix Cyathulae, the dried roots of *Cyathula capitata* Moquin-Tandon (Amaranthaceae), is the first ecdysterol possessing the C_{29} stigmastane skeleton. It has been clarified that cyasterone exhibits a strong metamorphosing hormone activity in the insect tests,[1,2] *inter alia*, it is over 30 times as active as the natural hormone ecdysterone in the Cynthia silkworm (*Samia cynthia*) test,[3] and it shows the highest anabolic activity in the mouse.[4]

We previously deduced the structure together with part of the stereochemistry of cyasterone as shown in formula I,[1] a rigid proof for the complete stereostructure being required. Our interests in the structure-activity relationship of ecdysterols prompted us to investigate the absolute configuration and the present report provides evidence that cyasterone is represented by stereoformula II.†

Since the stereochemistry of the cyasterone nucleus was previously suggested mainly by the physico-chemical evidence, a more conclusive evidence for the nucleus structure was obtained by the following manner.[3] Selective acetylation of cyasterone yielded the monoacetate (III) along with the 2,3,22-triacetate (IV). The mass spectrum of the monoacetate (III) exhibits, together with the molecular ion peak at *m/e* 562, the peaks at *m/e* 405, 387 and 369 attributed to the nucleus fragments which are 42 mass units higher than the corresponding peaks in that of cyasterone, and the peaks at *m/e* 201, 183, 157 and 113 due to the side-chain fragments which are consistent with those of cyasterone. This finding indicates that either of the OH groups at C–2 and C–3 was acetylated. Oxidation of the monoacetate (III) gave the cleaved products, the methyl ketone (V) and the aldehyde (VI). The former was identified as the known 2β-acetoxy-3β, 14α-dihydroxy-5β-pregn-7-ene-6, 20-dione,[5,6] and consequently the monoacetate (III) was deduced to be the 2-acetylated derivative. The sterostructure of the tetracycle of cyasterone was thus established.

The absolute configuration at C-20 and C-22 in cyasterone is identical with that of ecdysterone (VIII) and ponasterone A (IX) since: (1) Cyasterone and the ecdysterols

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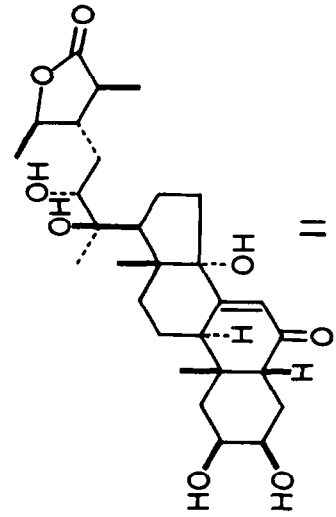
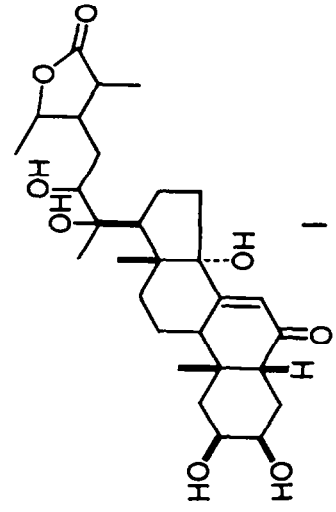
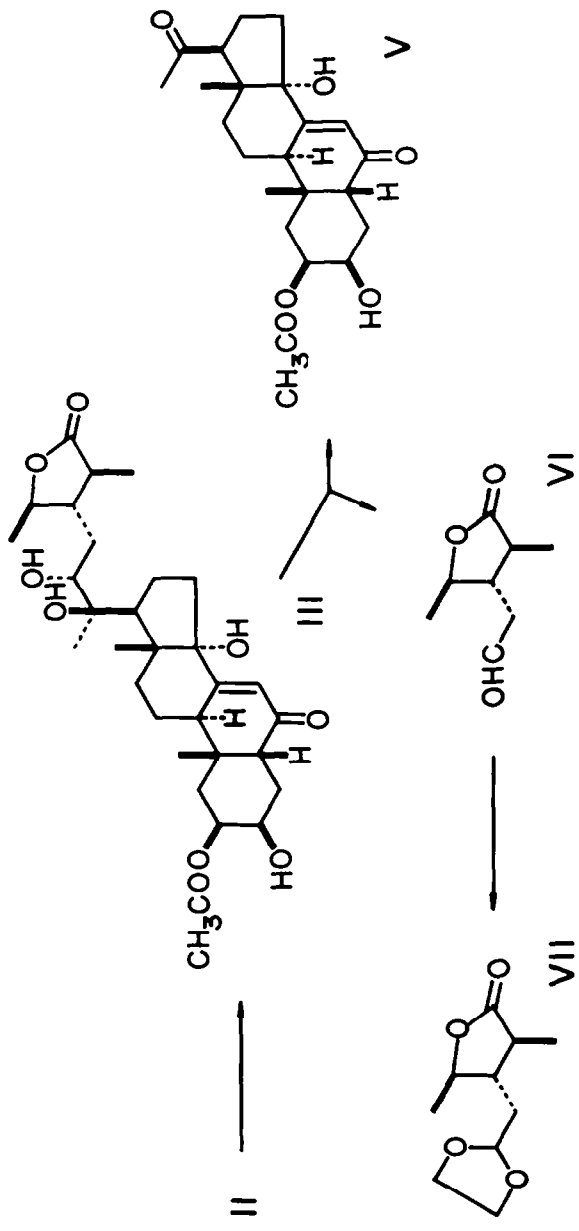
† Part of the material here presented has been outlined in a preliminary communication, *Chem. Pharm. Bull.* (Tokyo), **18**, 2132 (1970).



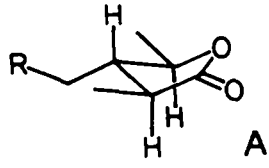



V
III
VI
VII
II

(VIII and IX) rapidly consume two molecules of periodate and readily give the 20,22-acetonide. (2) The chemical shifts of the C-18 and C-21 Me proton signals in the NMR spectra of cyasterone and its triacetate (IV) and the chemical shift and shape of the C-22 carbinyl proton signal in the spectrum of the triacetate (IV) are very similar to the corresponding data of the ecdysterols (VIII and IX) and their 2,3,22-triacetates[1] (Table), indicating that these hydrogens of cyasterone are situated in an environment similar to that of ecdysterone (VIII) and ponasterone A (IX). Since the C-20 and C-22 configurations in ecdysterone (VIII) and ponasterone A (IX) have already been established to be *R*,[7-10] those in cyasterone are consequently *R*.

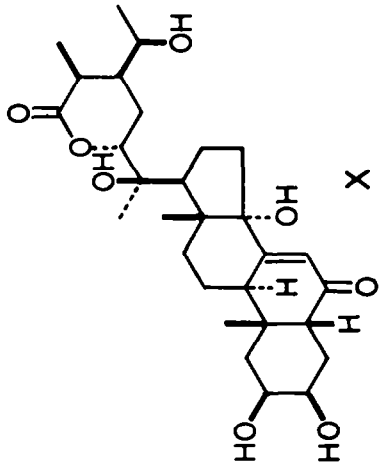
Quite recently, we have isolated another phytoecdysone, precyasterone (X), from the same plant source.[11] It has been shown that when precyasterone (X) is subjected to alkaline hydrolysis followed by acidification, δ-lactone ring opening and γ-lactone ring closure take place to give cyasterone, a fact which demonstrates that all the asymmetric centers in precyasterone and cyasterone have the same absolute configuration. Application of the Hudson-Klyne lactone rule[12] to precyasterone indicates that the absolute configuration at C-22 is *R*, confirming the above conclusion.

In order to solve the remaining problem, the sterochemistry of the γ-lactone moiety in the side-chain, the NMR spectrum of the side-chain fragment, the lactone aldehyde (VI),[1] was examined. When the expected decrease of the coupling constant between the C-24 and C-28 hydrogens* due to the electronegativity of the oxygen function at C-28 is taken into consideration, the observed *J* value (8Hz) demonstrated that these hydrogens are located in the quasiaxial-quasiaxial relationship.[13] On the other hand, the large observed value (11 Hz) for the coupling constant between the C-24 and C-25 Hydrogens is also consistent with a diquasiazial coupling.[14] Therefore, the C-25 Me group must be quasiequatorially situated. The orientation of the C-25 Me group was further investigated by the solvent effect of the C-27 Me proton signal on the adjacent C-26 lactonic CO group. In order to determine the solvent induced shift by the lactonic CO group alone, the influence of the aldehyde CO group in the lactone-aldehyde (VI) had to be eliminated by transformation into the acetal (VII). This was prepared from the lactone-aldehyde (VI) by treatment with 1,2-ethanediol in benzene in the presence of *p*-toluenesulphonic acid. The mass spectrum of the acetal (VII) exhibits the expected parent peak at *m/e* 200, and the IR and NMR spectra display no aldehyde absorption, while the NMR spectrum shows a 4H multiplet centered at 3·86 ppm originating from two methylene groups flanked by two O atoms. Now, it was found that the solvent-induced shifts for the C-27 Me resonance adjacent to the C-26 CO function suffered on passing from chloroform to benzene solution and from chloroform to pyridine solution, are +0·21 and −0·03 ppm, respectively, confirming that the C-25 Me group is located in the quasiequatorial orientation.[15] Consequently, the end of the side-chain must be represented by formula A or its enantiomer. A decision in favor of the part structure A for the lactone system was

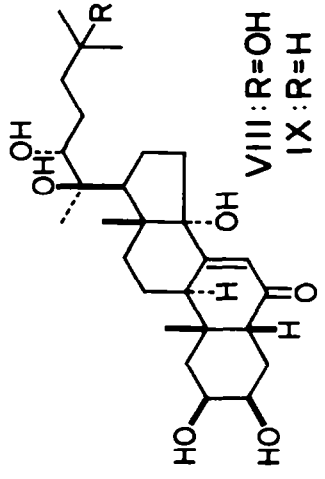


A

* Numbering of the C atoms of the parent substance is expediently retained.



X



VIII : R=OH
IX : R=H

made on the following evidence. Thus the optical rotations of the lactone (VII) were measured in methanol solution in the absence and presence of alkali. As a result, it was observed that the lactone (VII) is more dextrorotatory than the corresponding hydroxy acid. In applying the Hudson–Klyne lactone rule,[12] the absolute configuration at C-28 was established to be *R*, the absolute configurations at C-24 and C-25 being consequently deduced to be both *S* as shown in formula A. This conclusion was further corroborated by the circular dichroism curve of the lactone (VII) which exhibits a positive Cotton effect at 218 nm, indicating the β-configuration of the C-25 Me group.[16]

TABLE. PROTON SIGNALS[a]

	C-18	C-21	C-22
Ecdysterone (VIII)[b]	1·19s	1·55s	3·83dd
Ponasterone A (IX)[b]	1·16s	1·51s	3·74dd
Cyasterone (I)[b]	1·19s	1·51s	3·90[d]
Ecdysterone 2,3,22-triacetate[c]	0·85s	1·24s	4·82dd
Ponasterone A 2,3,22-triacetate[c]	0·85s	1·24s	4·79dd
Cyasterone 2,3,22-triacetate[c]	0·85s	1·24s	4·98[d]

[a] Abbreviations: s = singlet, dd = doublet of doublets
[b] in C_5D_5N [c] in $CDCl_3$
[d] Although the coupling constants are unclear due to overlapping of signals, the signal shape quite resembles those of the other congeners.

On the basis of the above evidence, it was concluded that cyasterone possesses the stereostructure II.

The absolute configuration at C-24 of cyasterone is identical with that of β-sitosterol,[17] a fact which is consistent with the most probable assumption that cyasterone is biosynthesized from β-sitosterol which is also found in the same plant source.

EXPERIMENTAL

M.ps are uncorrected. NMR spectra were measured on a Hitachi H-60 spectrometer. Chemical shifts are given in ppm units downfield from internal TMS and coupling constants (*J*) in Hz units. Abbreviations: s = singlet, d = doublet, m = multiplet, dd = doublet of doublets, and br = broad.

Partial acetylation of cyasterone. To cyasterone (300 mg) in pyridine (3 ml) and chloroform (12 ml) was added Ac_2O (3 ml) and the mixture was allowed to stand at room temp for 3 hr. After treatment in the usual manner, the product (330 mg) was chromatographed on silica gel (15 g).

Benzene–AcOEt (3:1) eluate (52 mg) was crystallized from MeOH to give IV[1] as colourless needles (34 mg), m.p. 250–252°; IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1776 (γ-lactone), 1742, 1242 (acetoxyl), 1657 (cyclohexenone).

Benzene–AcOEt (1:1) eluate (73 mg) was crystallized from MeOH to furnish *cyasterone 2-acetate* (III) as colourless needles (49 mg), m.p. 260–262°: MS *m/e*: 562 (M^+), 405 (M^+-157), 387 (M^+-157-18), 369 (M^+-157-36), 345 (M^+-157-60), 327 (M^+-157-18-60), 309 (M^+-157-36-60), 201 (M^+-361), 183 (M^+-361-18), 157 (M^+-405), 113 (M^+-449); IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1755 (γ-lactone), 1742, 1240 (acetoxyl), 1655 (cyclohexenone); NMR (C_5H_5N): 3H s at 1·08 ($C_{(19)}\underline{H}_3$), 3H s at 1·20 ($C_{(18)}\underline{H}_3$), 3H d at 1·35 (J = 7, $C_{(27)}\underline{H}_3$), 3H d at 1·35 (J = 7, $C_{(29)}\underline{H}_3$), 3H s at 1·54 ($C_{(24)}\underline{H}_3$), 3H s at 1·96 ($C\underline{H}_3COO$—), 1H m at 3·61 ($C_{(9)}\underline{H}$), 2H m in the range 3·75–4·15 ($C_{(22)}\underline{H}$, $C_{(28)}\underline{H}$), 1H br s at 4·25 ($C_{(3)}\underline{H}$), 1H br s at 6·23 ($C_{(7)}\underline{H}$).

AcOEt eluate (68 mg) was crystallized from MeOH to afford the recovered cyasterone as colourless needles (55 mg), m.p. 162–164°; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1752 (γ-lactone), 1650 (cyclohexenone).

Periodate oxidation of cyasterone 2-acetate. To III (30 mg) in MeOH (3 ml) was added $NaIO_4$ (30 mg) in water (2 ml). The mixture was left at room temp for 3 hr, poured into water, and extracted with AcOEt. After evaporation of the solvent, the residue (25 mg) was chromatographed over silica gel (3 g).

Elution with $CHCl_3$ gave VI[1] as a colourless oil (4 mg); IR ν_{max}^{KBr} cm^{-1}: 2840, 2730, 1729 (aldehyde), 1770 (γ-lactone).

Elution with $CHCl_3$–AcOEt (1:1) (12 mg) and crystallization from MeOH gave V as colourless needles (7 mg), m.p. 236–238°; IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1738, 1240 (acetoxyl), 1710 (acetyl), 1645 (cyclohexenone). Identification was carried out in the usual criteria (m.p., mixed m.p. and IR).

Acetalization of 2,4-dimethyl-3-(2-oxoethyl)-4-butanolide. The Butanolide VI (150 mg) in benzene (20 containing *p*-toluenesulphonic acid (10 mg) and 1,2-ethanediol (80 mg) was heated on an oil bath for 6 hr. Ether was added to the mixture, and the ether layer washed with water, dried, and evaporated to afford *2,4-dimethyl-3-(2,2-ethylenedioxyethyl)-4-butanolide* (VII) as a colourless oil (120 mg), $[\alpha]_D$ +7·3° (*c* 0·480, MeOH). ORD (*c* 0·480, MeOH): $[\Phi]_{350}$ +90, $[\Phi]_{300}$ +170, $[\Phi]_{232}^{peak}$ +1870, $[\Phi]_{210}$ −1870, CD (*c* 0·480, MeOH); $[\theta]_{250}$ 0, $[\theta]_{218}^{max}$ +22·8 × 10^2, $[\theta]_{206}$ +14·4 × 10^2; MS *m/e*: 200 (M^+), 127 (M^+-73), 113 (M^+-87), 112 (M^+-88), 97 (M^+-103), 73 (M^+-127), 69 (M^+-131); UV λ_{max}^{MeOH} nm (log ε): 270 (1·00), 215 (1·90); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (γ-lactone); NMR ($CDCl_3$): 3H d at 1·27 (*J* = 7, $C_{(27)}\underline{H}_3$), 3H d at 1·41 (*J* = 6, $C_{(29)}\underline{H}_3$), 4H m at 3·86 (—O—$(C\underline{H}_2)_2$—O—). 1H m at 4·05 ($C_{(28)}\underline{H}$), 1H dd at 4·84 (*J* = 4, 4, $C_{(22)}\underline{H}$); NMR (C_6H_6): 3h d at 1·06 (*J* = 7, $C_{(27)}\underline{H}_3$), 3H d at 1·06 (*J* = 6, $C_{(29)}\underline{H}_3$), 4h m at 3·30 (—C—$(C\underline{H}_2)_2$—O—)' 1H m at 3·60 ($C_{(28)}\underline{H}$), 1H dd at 4·48 (*J* = 4, 4, ($C_{(22)}\underline{H}$); NMR (C_5H_5N): 3H d at 1·28 (*J* = J, $C_{(27)}\underline{H}_3$), 3H d at 1·33 (*J* = 6, $C_{(29)}\underline{H}_3$), 4H m at 3·79 (—O—$(C\underline{H}_2)_2$—O—). 1H m at 4·10 ($C_{(28)}\underline{H}$), 1H dd at 4·90 (*J* = 4, $C_{(22)}\underline{H}$).

Alkali treatment of the acetal. To the acetal (VII) in MeOH was added a trace amount of conc. KOH and left standing at room temp for 15 min giving the corresponding hydroxy acid, $[\alpha]_D$ −3·1° (*c* 0·480, MeOH (KOH).

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