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CYASTERONE, AN INSECT METAMORPHOSING SUBSTANCE FROM *CYATHULA CAPITATA*: STRUCTURE†

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Abstract—A novel C_{29} insect-metamorphosing substance, cyasterone, has been isolated from *Cyathula capitata* (Amaranthaceae). Chemical and physico-chemical studies of cyasterone and its derivatives (II–V), and in particular its transformation into 14α-hydroxy-2,3-seco-5β-pregn-7-ene-6,20-dione-2,3-dial (VI) and 2,4-dimethyl-3-(2-oxoethyl)-4-butanolide (VII) have established the structure of cyasterone as shown in formula I.

FOLLOWING the recent clarification of the nature of the active principles responsible for the metamorphosing phenomena in insects,[1-8] it has become recognized that active substances are widely distributed also in the plant kingdom as evidenced by the numerous reports.[9-20]

During the course of screening tests on vegetable material by means of bioassay, it was believed that a substance possessing insect metamorphosing hormone activity was present in 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. The polar fraction of the extract when subjected to alumina chromatography, resulted in the isolation of a novel C_{29} insect metamorphosing substance (0·02% yield from the crude drug), for which the name cyasterone is proposed.

This paper presents evidence for the structure together with the partial stereochemistry as shown in formula I for cyasterone.‡

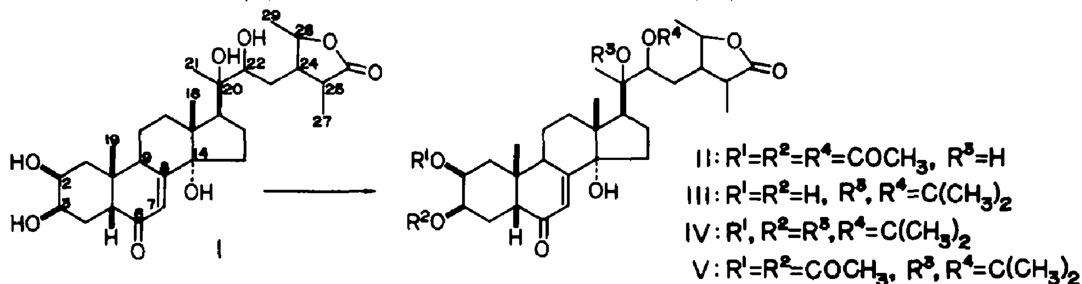
Since cyasterone is a polyhydroxy substance, as indicated by a strong OH absorption in the IR spectrum, it was difficult to prepare it free from water of crystallization and therefore, the analytical values for carbon and hydrogen varied with the drying conditions of the sample. The mass spectrum exhibited a peak corresponding to the molecular ion at *m/e* 520 and the correct molecular formula $C_{29}H_{44}O_8$ was consequently deduced. Upon drying the sample at high temperatures under reduced pressure the analytical data approximated the figures for a molecular formula $C_{29}H_{44}O_8 \cdot \frac{1}{2}H_2O$. The abnormally low m.p., 164–166°, of the semihydrate as a polyhydroxy steroid may be due to the water of crystallization. The molecular formula thus established was further confirmed by the elemental analyses of a number of cyasterone derivatives.

After establishment of the molecular formula, cyasterone was recognized as an insect metamorphosing substance, with a unique C_{29} skeleton.

† This paper forms Part I in the series on Steroids.

‡ Part of the material contained herein has been outlined in a preliminary communication: *Tetrahedron Letters* 3191 (1967).

Cyasterone was acetylated with acetic anhydride in pyridine at 100° for 1 hr to afford the triacetate (II). On treatment with acetone in the presence of *p*-toluenesulphonic acid cyasterone gave the monoacetonide (III) and the diacetonide (IV). When treated with hot aqueous ethanol the diacetonide (IV) was slowly hydrolysed to give the monoacetonide (III). Acetylation of the monoacetonide (III) yielded the monoacetonide diacetate (V). The formation of the diacetonide (IV) and the monoacetonide



diacetate (V) established the presence of four OH groups. As the diacetonide (IV) and the monoacetonide diacetate (V) still show IR absorption (KBr) due to an OH group (3485 and 3480 cm^{-1}, respectively), a fifth OH must be present. In the NMR spectrum of the triacetate (II) three signals for three hydrogens on carbons carrying the acetoxyl groups are visible at ca. 4·98, ca. 5·01, and 5·31 ppm, the presence of three secondary OH groups in cyasterone being proved. Since no more signals attributable to hydrogens on oxygen-bearing carbons were observed, the remaining two OH groups must be tertiary. Introduction of two acetyls upon acetylation of the monoacetonide (III) indicates that a secondary and a tertiary OH group are involved in the first acetonide formation. This was confirmed by the NMR spectrum of the monoacetonide diacetate (V) in which two signals arising from hydrogens on carbons attached to the two secondary acetoxyls occur in lower fields (4·98 and 5·25 ppm), whereas a signal originating from a hydrogen on carbon bearing the acetonide forming OH appear in a higher field (3·65 ppm). Since the diacetonide (IV) gave no acetylated product, the formation of the second acetonide linkage includes the remaining two secondary OH groups. From these observations, the presence of three secondary OH and two tertiary OH groups became certain. The IR spectra (KBr) of cyasterone and its derivatives II–V indicate the presence of an enone system in a 6-membered or larger ring (1667–1650 cm^{-1}) and a γ-lactone system (1778–1752 cm^{-1}), which is another unique feature of cyasterone. The eight O atoms can thus be satisfactorily accommodated.

All the accumulated data together with the high biological activity of cyasterone in the insect test clearly point to the general similarity between cyasterone and the hitherto known insect-metamorphosing substances, i.e. ecdysone (IX),[2] ponasterone A (X),[9] pterosterone (XI),[21] ecdysterone (XII),[4,5] inokosterone (XIII),[11] and 20,26-dihydroxyecdysone (XIV).[8] Therefore, the following structural elucidation of cyasterone has been based on the known metamorphosing substances.

The IR absorption (KBr) at 1650 cm^{-1}, the UV absorption at 243 mμ (log ε 4·11), and the NMR signal at 6·28 ppm shown by cyasterone indicate the presence of a β,β-disubstituted α,β-unsaturated ketone system. The UV maximum, after treatment with hot ethanolic hydrochloric acid solution, shows a shift to longer wavelength

giving two new maxima at 245 and 295 mμ. This observation is in accord with ecdysone which undergoes a similar change in the presence of acid—the elimination of the C-14 OH group and resulting formation of two isomeric dienones, the 8,14-dien-6-one and 7,14-dien-6-one derivatives, having the UV maxima at 244 and 293 mμ, respectively.[2] Thus, cyasterone probably has the steroid nucleus with the 7-en-6-one chromophore and the 14-OH group.

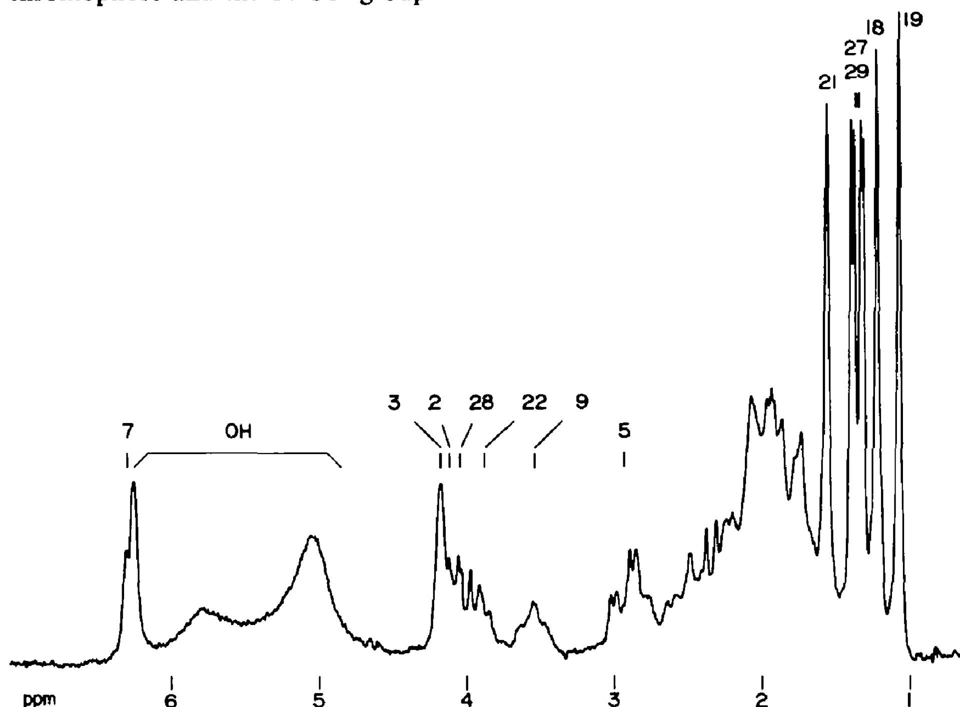
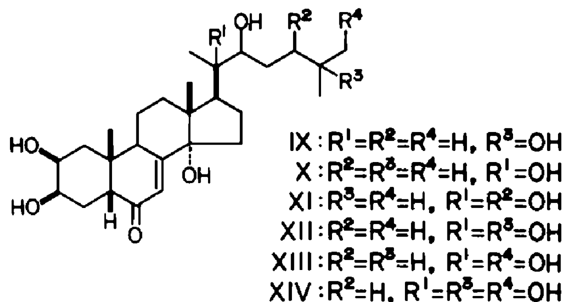


FIG. 1 NMR spectrum of cyasterone (C_5D_5N, 100 Mc/s).

The NMR spectra of cyasterone and ecdysterone in pyridine are similar (Fig. 1, Table 1). Cyasterone exhibits three Me singlets in similar regions to the C-18, C-19, and C-21 Me resonances of ecdysterone. However, the absence of a 6-proton singlet at 1·34 ppm attributed in the spectrum of ecdysterone to the C-26 and C-27 Me protons, and instead the presence of a 6-proton doublet at 1·33 ppm in cyasterone is observed.

TABLE 1. METHYL CHEMICAL SHIFTS (PYRIDINE)

		C-18	C-19	C-21	C-26	C-27	C-29
Ecdysone	(IX)	0·74	1·07	1·28	1·38	1·38	—
Ponasterone A	(X)	1·16	1·03	1·51	0·82	0·82	—
Pterosterone	(XI)	1·18	1·05	1·54	1·00	1·00	—
Ecdysterone	(XII)	1·19	1·06	1·55	1·34	1·34	—
Inokosterone	(XIII)	1·19	1·07	1·52	—	1·03	—
20,26-dihydroxyecdysone	(XIV)	1·22	1·08	1·58	—	1·48	—
Cyasterone	(I)	1·19	1·06	1·51	—	1·33	1·33



Comparison of the NMR spectra of cyasterone triacetate (II) and ecdysterone triacetate was more informative (Table 2). Thus the chemical shifts and splitting patterns of certain signals of cyasterone triacetate (II) coincide remarkably with those of the signals for the protons on carbons involved in the steroid nucleus of ecdysterone

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
Ecdysterone 2,3,22-triacetate	5·04 ddd	5·31 ddd	5·85 d	3·10 ddd	0·85 s	1·02 s	1·24 s	4·79 dd	1·18 s	1·21 s	—	—
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
Cyasterone monoacetonide diacetate	4·98 ddd	5·25 ddd	5·78 d	3·03 ddd	0·77 s	0·99 s	1·23 s	3·65 dd	—	1·24 d	4·10 dq	1·39 d

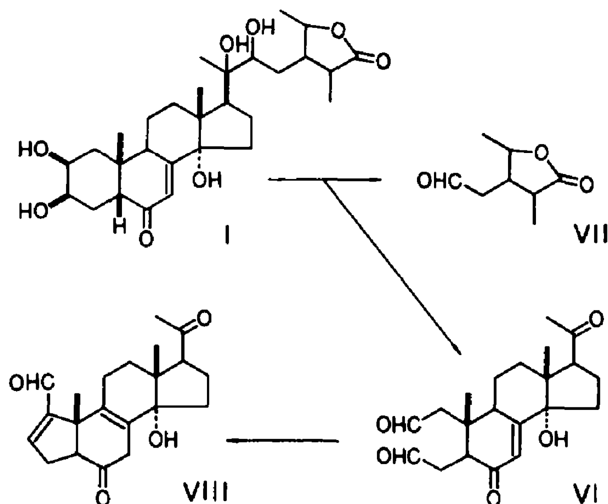
† Patterns are not clear due to overlapping of the signals.

triacetate. The patterns of the signals at ca. 5·01 and ca. 4·98 ppm in the spectrum of cyasterone triacetate (II) are not clear due to overlapping of both signals, but they are distinctly observed in the spectrum of the monoacetonide diacetate (V), since the latter signal is displaced to 3·65 ppm (Table 2). However, the spectra differ markedly in the signals for terminals of the side chains. Thus the absence of the two 3-proton signals at 1·18 and 1·21 ppm associated in the spectrum of ecdysterone triacetate with the C-26 and C-27 Me protons and the presence of two 3-proton doublets at 1·28 and 1·41 ppm as well as a 1-proton doublet of quadruplets at 4·10 ppm in cyasterone triacetate (II) is noted. Double resonance experiments revealed that the Me protons at 1·41 ppm are spin-coupled with the proton at 4·10 ppm ($J = 6$ c/s) which is further coupled with another proton ($J = 7$ c/s). This observation together with the presence of a lactone system indicate that a $>CH—CH(CH_3)—O—CO—$ moiety is present in cyasterone. As noticed above, cyasterone triacetate (II) shows the (C-22) carbonyl proton signal at ca. 4·98 ppm which is 0·19 ppm further downfield than the C-22 proton signal of ecdysterone triacetate, suggesting that the cyasterone structure has functional groups at some positions such that they produce a downfield shift in the C-22 hydrogen signal. They have later been ascribed to the previous lactone system.

On the basis of the above facts, the following conclusion can be drawn. Since the resonance frequencies of protons and especially of angular Me protons are sensitive to both the nature and orientation of substituent groups in the steroid nucleus, the close similarity of the chemical shifts and splitting patterns of certain NMR signals of cyasterone and ecdysterone, above described, demonstrates that both substances have a similar steroid nucleus with similarly oriented substituents. On the other hand, cystarone differs from ecdysterone in having two extra carbons. Indeed, the NMR and IR data show that, while ecdysterone has 5 carbon terminals, cyasterone possesses 6 carbon terminals in which two are accounted for as tertiary Me's, one as a tertiary Me on an OH-bearing carbon, one as a secondary Me, one as a secondary Me attached to a tertiary carbon carrying a lactonic oxygen, and the last one as a lactonic carbonyl (cf. Table 1 and 2); all of which indicates that the difference between these two substances lies in the structure of their side chains.

As stated above, cyasterone has five OH groups, in which a set of two secondary OH's and another set of one secondary OH and one tertiary OH participate in the diacetonide formation. The environment of the first glycol consisting of the two secondary OH's was clarified by NMR analysis with the aid of double resonance experiments. The glycol could be readily detected from two carbinyl proton signals appearing at 4·98 and 5·25 ppm in the spectrum of the monoacetonide diacetate (V). Decoupling experiments exhibited that both protons are spin-coupled to each other (J = 3 c/s) and each proton is further coupled to adjacent methylene protons, demonstrating the presence of a —CH_2—CH(OH)—CH(OH)—CH_2— system which can only be placed at C-1–C-4 in the steroid skeleton. The other glycol contains only one secondary OH group. The carbinyl proton signal in the spectrum of the monoacetonide diacetate (V) occurs at 3·65 ppm as a doublet of doublets ($J_1 = 9, J_2 = 3$ c/s). Since another OH group comprising the glycol is attached to a quaternary carbon bearing a Me, the glycol is involved in a —C(OH)(CH_3)—CH(OH)—CH_2— moiety, provided that these two OH's constitute a 1,2-glycol. This part structure can only be laid at C-20–C-23 in the steroid skeleton.

The nature of two glycol systems in cyasterone was next examined chemically by periodate oxidation, when two molecules of the reagent were consumed at a fairly rapid rate, i.e. in less than 1 hr. The presence of two 1,2-glycols and furthermore their *cis*-configurations can thus be confirmed. The product was separated by silica gel chromatography into the acetyl dialdehyde (VI) and the aldehyde (VII). The spectral properties of the oily dialdehyde (VI) showed the presence of two tertiary Me's (0·63, 1·25 ppm), an acetyl (1705 cm^{-1}, 2·13 ppm), a cyclohexenone (1662 cm^{-1}), two aldehyde (1728 cm^{-1}, 9·8–9·9 ppm), and a tertiary OH (3440 cm^{-1}). Comparison of the acetyl dialdehyde (VI) with that obtained from ecdysterone by periodate oxidation proved their identity. It was further converted by treatment with silica gel into the enal (VIII). The UV, IR and NMR data of the enal (VIII) demonstrated that it has two tertiary Me's (0·90, 1·21 ppm), an acetyl (1700 or 1708 cm^{-1}), a cyclohexanone (1708 or 1700 cm^{-1}), an α,β-unsaturated aldehyde (227 mμ, 2830, 2730, 1676, 1620 cm^{-1}, 9·91 ppm), and a tertiary OH (3540 cm^{-1}). Therefore, it is evident that, during silica gel treatment of the acetyl dialdehyde (VI), the two aldehyde functions suffer intramolecular aldol condensation and the ethylene bond at C-7 : C-8 migrates to C-8 : C-9, though it is not certain if the condensation proceeds as shown or *vice versa*. The enal (VIII) thus obtained was identified as the enal prepared from



ecdysterone by the same methods, establishing the identity of both cyasterone and ecdysterone in the structure of their steroid nuclei. The aldehyde (VII) was characterized as a 2,4-dinitrophenylhydrazone of mol wt 336 determined mass-spectrometry. The IR spectrum shows the presence of an aldehyde group (2840, 2730, 1729 cm^{-1}) and a γ-lactone system (1770 cm^{-1}). All the NMR signals were analyzed thoroughly with the aid of double resonance experiments (Fig. 2): the aldehyde proton (9·81 ppm) is coupled (J = 1·5 c/s) with the methylene protons (2·66 ppm) which are further

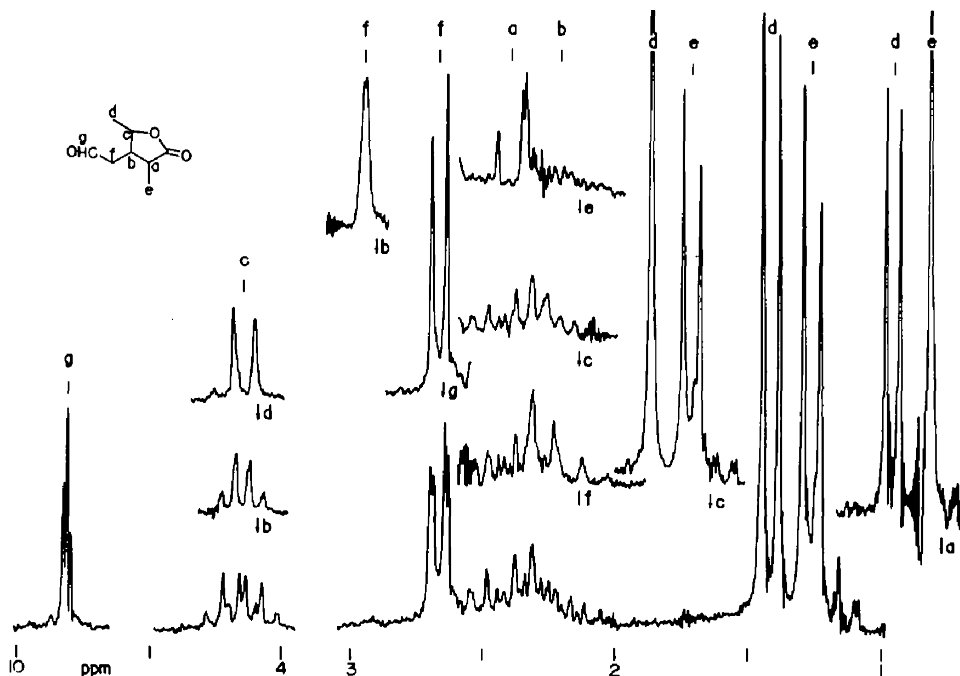


FIG. 2 NMR spectrum of 2,4-dimethyl-3-(2-oxoethyl)-4-butanolide ($CDCl_3$, 100 Mc/s). Abbreviation: ↓a = irradiation at H_a.

spin-coupled (J = 6 c/s) to the methine proton (2·19 ppm). The latter is, in addition, coupled (J = 11 and 8 c/s, respectively) with the methine proton (2·36 ppm) and the carbinyl proton (4·15 ppm). The last two protons are respectively further spin-coupled (J = 7 and 6 c/s) with the Me protons (1·26 and 1·41 ppm). From these spectral properties, the structure VII can be deduced for the lactono aldehyde. Arrangement of the two fragments VI and VII leads to the conclusion that cyasterone is represented by formula I exclusive of stereochemistry.

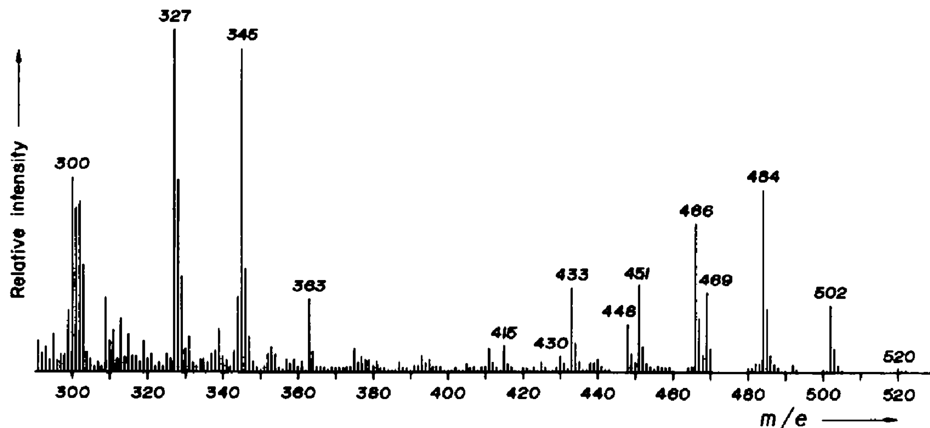


FIG. 3 Mass spectrum of cyasterone (70 eV).

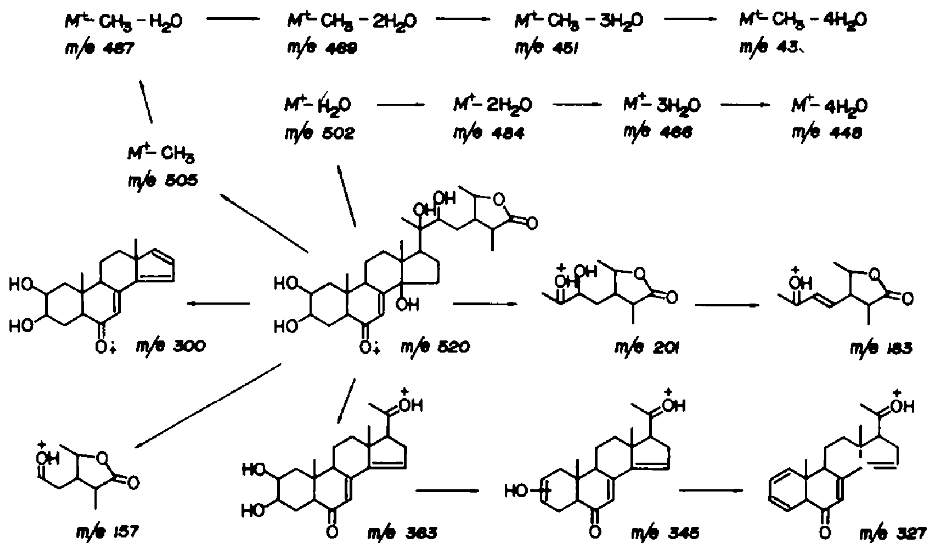


FIG. 4 Principal mass fragmentation of cyasterone.

The mass spectral data of cyasterone fully support the assigned structure I as discussed below. Because of the many OH groups and, consequently, a very low volatility, cyasterone itself is not neccessarily suitable for mass spectral measurements. Therefore, it gave peaks of only small intensities especially in the high mass region. The intensity of the parent peak at m/e 520 is very weak (0·4% relative to the

base peak at *m/e* 43). Instead, the tendency of this highly hydroxylated substance to eliminate water gave the characteristic peaks at *m/e* 502 (M-18, 6%), 484 (M-36, 16%), 466 (M-54, 13%), 448 (M-72, 4%), 430 (M-90, 1%), which correspond to the loss of 1–5 molecules of water. The peak at *m/e* 505 (M-15, 0·2%) shows loss of Me, and the peaks at *m/e* 487 (M-15-18, 1%), 469 (M-15-36, 7%), 451 (M-15-54, 7%), 433 (M-15-72, 8%), and 415 (M-15-90, 2%) indicate that up to 5 molecules of water can further be eliminated from the ion (M-15). The major peaks at *m/e* 363 (M-157, 7%), 345 (M-157-18, 29%), and 327 (M-157-36, 30%) are attributed to the loss of the side-chain by the fission of the C-20:C-22 bond without rearrangement which is expected of a vicinal diol, and, therefore, are consistent with the presence of the C-20 and C-22 OH groups. The prominent peaks at *m/e* 363, 345, and 327 are also present in the spectrum of ecdysterone and indicate that the fragments remaining after loss of the side chain are the same in both substances and consequently, the difference between these two substances lies in the side chain. This finding was further confirmed by the peak at *m/e* 300 (M-220, 17%) showing the complete loss of the side chain at C-17 and a subsequent loss of one molecule of water and hydrogen transfer. This fragmentation is also observed in ecdysterone. The major peaks at *m/e* 201 (M-319, 9%) and 183 (M-319-18, 28%) are the expected side-chain fragments for structure I and disclose the C-17:C-20 bond cleavage without rearrangement. The intense peak at *m/e* 157 (M-363, 45%) which agrees with the side-chain C-20:C-22 bond fragmentation also favors the structure I for cyasterone.

Concerning the stereochemistry of cyasterone, since the periodate oxidation product (VI) is identical with that of ecdysterone (XII), the stereostructure of the nucleus of cyasterone must be the same as that of ecdysterone (XII) except for the configurations at C-2, 3, and 5.

The A/B *cis*-ring fusion was derived from the fact that cyasterone gave an ORD curve of *a* +65 and a CD curve of molecular elipticity $+43 \times 10^2$ for the $n \rightarrow \pi^*$ transition centered at 338 mμ. The former was consistent with that of A/B *cis* Δ^7-6-keto steroids (*a* + 60) but not with that of A/B *trans* Δ^7-6-keto steroids (*a* + 120–160).[22] Furthermore, the A/B *cis* and *trans* isomers of methyl 2β,3β,14α-trihydroxy-23,24-bisnor-chol-7-en-6-on-22-oate are known to exhibit their C-19 Me proton signals at fairly separate positions; i.e. 1·01 and 1·29 ppm, respectively.[23] Therefore, the remarkable coincidence of the line positions of the C-19 Me proton signals of cyasterone and ecdysone (IX) (Table 1) are consistent with the A/B *cis*-ring junction of cyasterone.

As mentioned, the rapid consumption of periodate by cyasterone suggested the *cis*-configuration of the two glycols. The *cis*-configuration of the glycol in the A-ring was further confirmed by NMR analysis of the monoacetonide diacetate (V). The coupling constant (3 c/s) between the two carbinyl protons at 4·98 and 5·25 ppm arising from the C-2 and C-3 hydrogens or *vice versa* demonstrates an equatorial–equatorial or axial–equatorial relation for these two hydrogens on a cyclohexane ring in a chair form as judged from the Karplus equation. The small couplings of the hydrogen at 5·25 ppm with the neighboring methylenic hydrogens indicate an equatorial–equatorial and an equatorial–axial relationship, showing an equatorial orientation for the hydrogen. The coupling constants (4 and 12 c/s) between the hydrogen at 4·98 ppm and the adjacent methylene hydrogens are compatible with axial–equatorial and axial–axial splittings, respectively, a fact which demonstrates

the hydrogen to be axially-situated. The combined evidence also points to the *cis*-configuration of the glycol. Although the conformation of the glycol thus deduced is in accordance with either a 2β,3β-dihydroxy or 2α,3α-dihydroxy structure, the remarkable coincidence of the chemical shifts of the C-19 Me protons of cyasterone and ecdysone (IX) (Table 1) suggests the β-*cis* glycol structure.

These observations indicate the stereochemistry in the nucleus of cyasterone to be as shown in formula I. More detailed investigation on the stereo-structure of cyasterone is now in progress.

Cyasterone was assayed in terms of its ability to provoke puparium formation of isolated larval abdomens of two kinds of insects. As a result, it was observed that, in the house-fly (*Musca domestica*) test, carried out by Kobayashi,[24] cyasterone exhibited the activity of the same order as that of ecdysterone (Table 3), and in the

TABLE 3. ASSAY OF CYASTERONE ON ISOLATED ABDOMENS OF THE HOUSE-FLY, *Musca domestica*

Dose (μg in 10% ethanol (5 μl)/animal)	Number of test animals	Number of pupation	Judgement
5	15	15	positive
0·5	15	10	positive
0·05	15	11	positive
0·005	15	0	
0·0025	15	3	
0	20	0	

blowfly (*Sarcophaga peregrina*) test, carried out by Ohtaki *et al.*,[25] it showed the slightly higher activity (1.2) relative to ecdysterone. Cyasterone was also assayed on its activity upon the induction of imaginal development of dauer pupae (artificially made diapansing brainless pupae) of two kinds of insects. Thus, it was found that, in the silkworm (*Bombyx mori*) test, performed by Kobayashi,[24] cyasterone is over 5 times as active as ecdysterone, while in the Cynthis silkworm (*Samia cynthia*) test, performed by Williams,[26] cyasterone is over 30 times as active as ecdysterone. Cyasterone was further tested with respect to its potency to accelerate the protein anabolism in mouse liver. In consequence, it was shown that cyasterone, as with the other metamorphosing substances (i.e. ponasterone A, pterosterone, ecdysterone and inokosterone), exhibits a high stimulating activity on protein synthesis.[27] The tonic effect claimed for the crude drug, Radix Cyathulae, in Oriental medicine, may be due to the anabolic activity of its constituent, cyasterone.

EXPERIMENTAL

M.ps are uncorrected. Specific rotations refer to $CHCl_3$ soln unless specified to the contrary. ORD and CD curves were recorded in dioxan soln. NMR spectra were determined on Varian HA-100 spectrometers in $CDCl_3$ soln unless otherwise indicated. Chemical shifts are expressed in ppm downfield from internal TMS and coupling constants (J) in c/s. Abbreviations: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, dd = doublet of doublets, and br = broad.

Isolation of cyasterone. 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 *cyasterone* (I) as colourless needles (4·0 g), m.p. 164–166°, $[\alpha]_D$ +64·5° (*c* 8·6, pyridine), ORD (*c* 0·100): $[\phi]_{359}^{peak}$ +3400, $[\phi]_{308}^{trough}$ −3100, CD (*c* 0·100): $[\theta]_{338}$ +43 × 10^2. UV λ_{max}^{EtOH} mμ (log ε): 243 (4·11), 305 (2·49). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1752 (γ-lactone), 1650 (cyclohexenone); NMR (C_5H_5N): 3H s at 1·06 ($C_{(19)}H_3$), 3H s at 1·19 ($C_{(18)}H_3$), 6H d at 1·33 (*J* = 6, $C_{(27)}H_3$, $C_{(29)}H_3$), 3H s at 1·51 ($C_{(21)}H_3$), 1H br dq at 3·50 ($C_{(9)}H$), 3H m at 3·8–4·1 ($C_{(22)}H$, $C_{(28)}H$, $C_{(2)}H$), 1H br s at 4·12 ($C_{(3)}H$), 1H d at 6·28 (*J* = 2, $C_{(7)}H$). (Found: C, 65·51; H, 8·65. $C_{29}H_{44}O_8 \cdot \frac{1}{2}H_2O$ requires: C, 65·76; H, 8·56%). Liebermann–Burchard reaction: positive (red).

Acetylation of cyasterone. Cyasterone (200 mg) in Ac_2O (1 ml) and pyridine (2 ml) was heated at 100° for 1 hr. The mixture was diluted with water and warmed to decompose the excess Ac_2O. Deposited crystals were collected by filtration and crystallized from MeOH giving *cyasterone* 2,3,22-*triacetate* (II) as colorless needles, m.p. 251–252°, $[\alpha]_D$ +69·0° (*c* 2·6), ORD (*c* 0·099): $[\phi]_{360}^{peak}$ +4200, $[\phi]_{310}^{trough}$ −2300, CD (*c* 0·099): $[\theta]_{339}$ +55 × 10^2; UV λ_{max}^{EtOH} mμ (log ε): 243 (4·11), 309 (2·20). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1776 (γ-lactone), 1742, 1242 (acetoxyl), 1657 (cyclohexenone); NMR 3H s at 0·85 ($C_{(18)}H_3$), 3H s at 1·02 ($C_{(19)}H_3$), 3H s at 1·25 ($C_{(21)}H_3$), 3H d at 1·28 (*J* = 8, $C_{(27)}H_3$), 3H d at 1·41 (*J* = 6, $C_{(29)}H_3$), 3H s at 1·99, 2·09, 2·11 (CH_3COO—), 1H ddd at 3·11 (*J* = 2, 8, 10, $C_{(9)}H$), 1H dq at 4·10 (*J* = 7, 6, $C_{(28)}H$), 1H m at ~4·98 ($C_{(22)}H$), 1H m at ~5·01 ($C_{(2)}H$), 1H ddd at 5·31 (*J* = 3, 4, 5, $C_{(3)}H$), 1H d at 5·85 (*J* = 2, $C_{(7)}H$). (Found: C, 64·60; H, 7·99. $C_{35}H_{50}O_{11}$ requires: C, 64·99; H, 7·79%).

Acetonide formation of cyasterone. Cyasterone (200 mg) in acetone (20 ml) containing *p*-toluenesulphonic acid (200 mg) was stirred at room temp for 2 hr. The mixture was diluted with water and extracted with AcOEt. The product (210 mg) was chromatographed over silica gel (10 g).

$CHCl_3$–ether (5:1) eluate (78 mg) was crystallized from MeOH to give *cyasterone diacetonide* (IV) as colorless needles, m.p. 212·5–213·5°, $[\alpha]_D$ +33·2° (*c* 0·146). IR ν_{max}^{KBr} cm^{-1}: 3485 (OH), 1773 (γ-lactone), 1662 (cyclohexenone). NMR: 3H s at 0·79 ($C_{(18)}H_3$), 3H s at 0·97 ($C_{(19)}H_3$), 3H d at 1·28 (*J* = 6, $C_{(27)}H_3$), 3H s at 1·32 ($C_{(21)}H_3$), 3H s at 1·32, 1·32, 1·41, 1·47 (—O—C(CH_3)$_2$—O—), 3H d at 1·44 (*J* = 6, $C_{(29)}H_3$), 1H br dd at 2·83 (*J* = 9, 8, $C_{(9)}H$), 1H dd at 3·70 (*J* = 9, 3, $C_{(22)}H$), 3H m at 4·1–4·3 ($C_{(2)}H$, $C_{(3)}H$, $C_{(28)}H$), 1H d at 5·81 (*J* = 2, $C_{(7)}H$). (Found: C, 70·10; H, 8·78 $C_{35}H_{52}O_8$ requires: C, 69·97; H, 8·72%).

AcOEt eluate (130 mg) was crystallized from MeOH to yield *cyasterone monoacetonide* (III) as colorless needles, m.p. 277–278°, $[\alpha]_D$ +48·0° (*c* 5·3); UV λ_{max}^{EtOH} mμ (log ε): 243 (4·08), 310 (2·08); IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1760 (γ-lactone), 1655 (cyclohexenone); NMR: 3H s at 0·79 ($C_{(18)}H_3$), 3H s at 0·95 ($C_{(19)}H_3$), 3H d at 1·29 (*J* = 6, $C_{(27)}H_3$), 3H s at 1·32 ($C_{(21)}H_3$), 6H s at 1·41 (—O—C(CH_3)$_2$—O—), 3H d at 1·44 (*J* = 6, $C_{(29)}H_3$), 1H br s at 5·80 ($C_{(7)}H$). (Found: C, 66·63; H, 8·56. $C_{32}H_{48}O_8 \cdot H_2O$ requires: C, 66·41; H, 8·71%).

Partial hydrolysis of cyasterone diacetonide. Cyasterone diacetonide IV (30 mg) in aqueous EtOH (EtOH–water = 3:1) (2 ml) was heated under reflux for 5 hr. After AcOEt extraction, the product was crystallized from MeOH to afford the monoacetonide (III) as colorless needles, m.p. 277–278°; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1760 (γ-lactone), 1655 (cyclohexenone). Identification was carried out in the usual criteria.

Acetylation of cyasterone monoacetonide. The monoacetonide III (67 mg) in Ac_2O (0·15 ml) and pyridine (0·3 ml) was heated at 100° for 1 hr. The mixture was diluted with water, warmed to decompose the excess Ac_2O, and cooled. After filtration, the collected crystals (70 mg) were crystallized from MeOH yielding *cyasterone monoacetonide diacetate* (V) as colorless needles, m.p. 251–253°, $[\alpha]_D$ +62·1° (*c* 0·245); UV λ_{max}^{EtOH} mμ (log ε): 242 (4·10), 310 (2·04); IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1778 (γ-lactone), 1742, 1244 (acetoxyl), 1667 (cyclohexenone); NMR: 3H s at 0·77 ($C_{(18)}H_3$), 3H s at 0·99 ($C_{(19)}H_3$), 3H s at 1·23 ($C_{(21)}H_3$), 3H d at 1·24 (*J* = 7, $C_{(27)}H_3$), 3H s at 1·30, 1·37 (—O—C(CH_3)$_2$—O—), 3H d at 1·39 (*J* = 6, $C_{(29)}H_3$), 3H s at 1·95, 2·05 (CH_3COO—), 1H ddd at 3·03 (*J* = 2, 8, 10, $C_{(9)}H$), 1H dd at 3·65 (*J* = 9, 3, $C_{(22)}H$), 1H dq at 4·10 (*J* = 8, 6, $C_{(28)}H$), 1H ddd at 4·98 (*J* = 4, 12, 3, $C_{(2)}H$), 1H ddd at 5·25 (*J* = 3, 4, 5, $C_{(3)}H$), 1H d at 5·78 (*J* = 2, $C_{(7)}H$). (Found: C, 67·32; H, 8·37. $C_{36}H_{52}O_{10}$ requires: C, 67·06; H, 8·13%).

Attempted acetylation of cyasterone diacetonide. Cyasterone diacetonide IV (3 mg) and Ac_2O (0·1 ml) in pyridine (0·2 ml) were heated at 100° for 1 hr. The mixture was diluted with water and the deposited crystals were collected and crystallized from MeOH to give the starting diacetonide (IV) as colorless needles, m.p. 211–213°. The identity was confirmed by the usual criteria.

Acid treatment of cyasterone. Cyasterone (0·2 mg) in dil HCl (conc HCl–EtOH = 1:100, 1 ml) was heated under reflux for 1 hr giving the mixture of the 8,14-dien-6-one and the 7,14-dien-6-one, UV $\lambda_{max}^{EtOH(HCl)}$ mμ: 245, 295.

Periodate oxidation of cyasterone. To cyasterone (121 mg) in MeOH (7 ml) was added $NaIO_4$ (120 mg) in water (8 ml) and the mixture was set aside at room temp. After 1 hr the product (100 mg) was isolated by AcOEt extraction and chromatographed on silica gel (4 g).

Elution with $CHCl_3$ gave *2,4-dimethyl-3-(2-oxoethyl)-4-butanolide* (VII) as a colorless oil (15 mg); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2840, 2730, 1729 (aldehyde), 1770 (γ-lactone); NMR: 3H d at 1·26 (J = 7, $C_{(26)}\underline{H}_3$†), 3H d at 1·41 (J = 6, $C_{(29)}\underline{H}_3$), 1H tdd at 2·20 (J = 6, 11, 8, $C_{(24)}\underline{H}$), 1H dq at 2·39 (J = 11, 7, $C_{(25)}\underline{H}$), 2H dd at 2·66 (J = 1·5, 6, $C_{(23)}\underline{H}_2$), 1H dq at 4·15 (J = 8, 6, $C_{(28)}\underline{H}$), 1H t at 9·81 (J = 1·5, $C_{(22)}\underline{H}$). 2,4-Dinitrophenylhydrazone, prepared in the customary manner (($(NO_2)_2$ $C_6H_3NHNH_2$—H_2SO_4—EtOH), crystallized from EtOH to give orange plates, m.p. 136·5–137·5°, MS *m/e*: 336 (parent peak).

Elution with AcOEt afforded *14α-hydroxy-2,3-seco-5β-pregn-7-ene-6,20-dione-2,3-dial* (VI) as a colorless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610, 3440 (OH), 1728 (aldehyde), 1705 (acetyl), 1662 (cyclohexenone); NMR (60 Mc/s): 3H s at 0·63 ($C_{(18)}\underline{H}_3$), 3H s at 1·25 ($C_{(19)}\underline{H}_3$), 3H at 2·13 ($C_{(21)}\underline{H}_3$), 1H s at 5·88 ($C_{(7)}\underline{H}$), 2H m at 9·8–9·9 ($C_{(2)}\underline{H}$, $C_{(3)}\underline{H}$).

Aldol condensation of dialdehyde. The dial VI was run on a thin-layer plate (silica gel, 1 mm thick) using AcOEt–hexane (4:1) as a eluant. The plate was heated at 110° for 10 min. Extraction of the appropriate band (R_f 0·35–0·05) with AcOEt and crystallization from AcOEt yielded the enal (VIII) as colorless needles, m.p. 197–200° (decomp.); UV λ_{max}^{EtOH} mμ (log ε): 228 (3·97); IR ν_{max}^{KBr} cm^{-1}: 3540 (OH), 2830, 2730, 1676, 1620 (enal), 1708, 1700 (acetyl, cyclohexanone); NMR (C_5D_5N + D_2O): 3H s at 0·84 ($C_{(18)}\underline{H}_3$), 3H s at 1·18 ($C_{(19)}\underline{H}_3$), 3H s at 2·11 ($C_{(21)}\underline{H}_3$), 1H br peak at 6·76 ($C_{(2)}\underline{H}$), 1H s at 9·91 (C$\underline{H}$O). The identity with the enal (VIII), obtained from ecdysterone by periodate oxidation followed by silica gel treatment,[11] was confirmed by the usual criteria.

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

REFERENCES

1. A. Butenandt and P. Karlson, *Z. Naturforschg.* **9b**, 389 (1954).
2. P. Karlson, H. Hoffmeister, H. Hummel, P. Hocks and G. Spitellar, *Chem. Ber.* **98**, 2394 (1965) and the preceding papers.
3. R. Huber and W. Hoppe, *Ibid.* **98**, 2403 (1965).
4. P Hocks and R Wiechert, *Tetrahedron Letters* 2989 (1966).
5. H. Hoffmeister and H. F. Grützmacher, *Ibid.* 4017 (1966); H. Hoffmeister, H. F. Grützmacher and K. Dünnebeil, *Z Naturforschg.* **22b**, 66 (1967)
6. D H. S. Horn, E. J. Middleton, J. A. Wunderlich and F Hampshire, *Chem. Comm.* 339 (1966).
7. J. N. Kaplanis, M. J. Thompson, R. T. Yamamoto, W. E. Robbins and S. J. Louloudes, *Steroids* **8**, 605 (1966).
8. M. J. Thompson, J. N Kaplanis, W. E. Robbins and R. T. Yamamoto, *Chem. Comm.* 650 (1967).
9. K. Nakanishi, M. Koreeda, S. Sasaki, M. L. Chang and H. Y. Hsu, *Ibid.* 915 (1966).
10. M N. Galbraith and D. H. S Horn, *Ibid.* 905 (1966).
11. T Takemoto, S. Ogawa and N. Nishimoto, *Yakugaku Zasshi* **87**, 325 (1967); **87**, 1469 (1967).
12. J. Jizba, V. Herout and F. Šorm, *Tetrahedron Letters* 1689 (1967).
13. H. Rimpler and G. Schulz, *Ibid.* 2033 (1967).
14. T Takemoto, S. Ogawa, N. Nishimoto, H. Hirayama and S. Taniguchi, *Yakugaku Zasshi* **87**, 748 (1967).
15. T. Takemoto, S. Ogawa, N. Nishimoto and H. Hoffmeister, *Z. Naturforschg.* **22b**, 481 (1967).
16. S. Imai, S. Fujioka, K. Nakanishi, M. Koreeda and T. Kurokawa, *Steroids* **10**, 557 (1967).
17. T. Takemoto, Y. Hikino, T. Arai, M. Kawahara, C. Konno, S. Arihara and H. Hikino, *Chem. Pharm. Bull.* **15**, 1815 (1967)

[18] T. Takemoto, S. Ogawa, N. Nishimoto and S. Taniguchi, *Yakugaku Zasshi* **87**, 1478 (1967).
[19] T. Takemoto, S. Ogawa, N. Nishimoto, K. Y. Yen, K. Abe, T. Sato, K. Osawa and M. Takahashi, *Ibid.* **87**, 1521 (1967).
[20] T. Takemoto, Y. Hikino, H. Jin and H. Hikino, *Ibid.*, **88**, 359 (1968).
[21] T. Takemoto, S. Arihara, Y. Hikino and H. Hikino, *Tetrahedron Letters* 375 (1968).
[22] K. Nakanishi, private communication. cf. Ref. 9.
[23] J. B. Shidall, J. P. Marshall, A. Bowers, A. D. Cross, J. A. Edwards and J. H. Fried, *J. Am. Chem. Soc.* **88**, 379 (1966).
[24] M. Kobayashi, private communication.
[25] T. Ohtaki, R. D. Milkman and C. M. Williams, *Proc. Nat. Acad. Sci.* **58**, 981 (1967).
[26] C. M. Williams, private communication.
[27] S. Okui, T. Otaka, M. Uchiyama, T. Takemoto, H. Hikino, S. Ogawa and N. Nishimoto, *Chem. Pharm. Bull.* **16**, 374 (1968).