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

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Following the recent clarification of the nature of the active principles responsible for the moulting phenomena in insects,[1-6] it has become generally recognized that active substances are widely distributed also in the plant kingdom; isolations of the ponasterones from Podocarpus nakaii,[7] crustecdysone from Podocarpus elatus,[8] and ecdysterone and inokosterone from Achyranthes fauriei[9] and Morus sp.[10] have been reported. During the course of screening tests on vegetable materials by means of Calliphora bioassay, the methanol extract of the roots of Cyathula capitata Moquin-Tandon (Amaranthaceae) was found to show moulting hormone activity. Alumina chromatography of the fraction soluble in ethyl acetate resulted in the isolation of a new active substance (0.02 % yield from the dried roots), for which the name cyasterone is proposed. This communication presents evidence leading to the structure I for cyasterone.

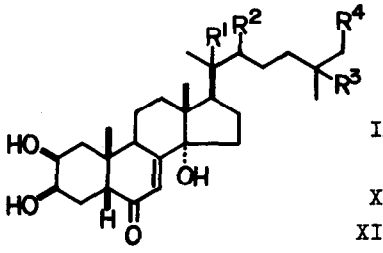
Cyasterone, $C_{29}H_{44}O_8 \cdot 1/2\ H_2O$,[*1] m.p. 164-166°, $[\alpha]_D$ +64.5° (pyridine), afforded the following derivatives: triacetate (II), $C_{35}H_{50}O_{11}$, m.p. 251-252°, monoacetonide (III), $C_{32}H_{48}O_8 \cdot H_2O$, m.p. 272-273°, diacetonide (IV), $C_{35}H_{52}O_8$, m.p. 212.5-213.5°, and the monoacetonide diacetate (V), m.p. 251-253°, establishing the presence of four hydroxyl groups. However, a fifth hydroxyl must be present, since the derivatives IV and V still show IR absorption (KBr) due to a hydroxyl group (3485-3480 cm^{-1}). The IR spectra (KBr) of cyasterone and its derivatives II ~ V indicate the presence of a γ-lactone system (1778-1752 cm^{-1}) and an enone system in a six-membered or larger ring (1667-1650 cm^{-1}). The eight oxygen atoms in the molecule can thus be satisfactorily accommodated.

The chemical shifts and splitting patterns of certain NMR signals[*2] of cyasterone triacetate (II) coincide remarkably with those of the corresponding signals of the triacetate of ecdysterone (X) (see Table I.). The CD curve of cyasterone ($[\theta]_{338}$ $+43 \times 10^2$, dioxan) is almost superimposable on that of ecdysterone (X) ($[\theta]_{338}$ $+45 \times 10^2$, dioxan). Treatment of cyasterone with hydro-

TABLE I. 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	1.02 s	0.85 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	1.02 s	0.85 s	1.25 s	~4.98 *	1.28 d	--	4.10 dq	1.41 d

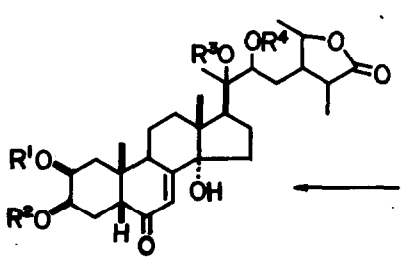
* Patterns are not clear due to overlapping of the signals, but distinctly observed in the spectrum of the monoacetonide diacetate (V).



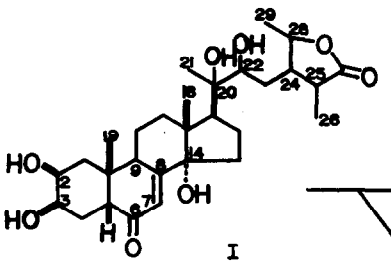
IX: $R^1=R^4=H$, $R^2=R^3=OH$
X: $R^1=R^2=R^3=OH$, $R^4=H$
XI: $R^1=R^2=OH$, $R^3=R^4=H$
XII: $R^1=R^2=R^4=OH$, $R^3=H$

TABLE II. Methyl chemical shifts (pyridine).

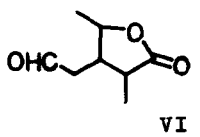
		C-18	C-19	C-21	C-26	C-27	C-29
Ecdysone	(IX)[2]	0.73	1.07	1.28d	1.38	1.38	--
Ecdysterone	(X)[5]	1.19	1.06	1.55	1.34	1.34	--
Ponasterone A	(XI)[9]	1.16	1.03	1.51	0.82d	0.82d	--
Inokosterone	(XII)[10]	1.19	1.07	1.52	1.03d	--	--
Cyasterone	(I)	1.19	1.06	1.51	1.33d	--	1.33d



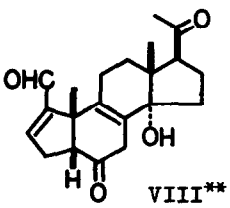
II: $R^1=R^2=R^4=COCH_3$, $R^3=H$
III: $R^1=R^2=H$, R^3,R^4=acetonide
IV: $R^1,R^2=R^3,R^4$=acetonide
V: $R^1=R^2=COCH_3$, R^3,R^4=acetonide



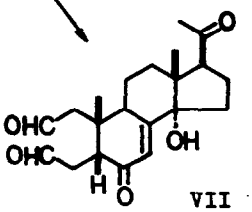
I



VI



VIII**



VII

** The structure in the A-ring is tentative (an alternative structure also possible).

chloric acid in methanol resulted in the disappearance of the starting material, which has UV maximum at 243 mμ (the 7-en-6-one chromophore), and the formation of products with UV maxima at 245 and 295 mμ (the 8,14-dien-6-one and 7,14-dien-6-one chromophores, respectively).[2] These observations suggest that cyasterone and ecdysterone (X) possess the same structure in steroid skeletons.

However, cyasterone differs from ecdysterone (X) in having two extra carbon atoms. Indeed, the NMR and IR data show that, while ecdysterone (X) has five carbon terminals, cyasterone possesses six carbon terminals in which two are accounted for as tertiary methyls, one as a tertiary methyl on a hydroxyl-bearing carbon, one as a secondary methyl, one as a secondary methyl attached to a tertiary carbon carrying a lactonic oxygen, and the last one as a lactonic carbonyl (cf. Table I. and II.); all of which indicates that the difference between these two substances lies in the structure of their side chains.

In order to establish the presence of two α-glycol systems, suggested by the formation of the diacetonide (IV), periodate oxidation of cyasterone was carried out. Two moles of the reagent were rapidly consumed and afforded the aldehyde (VI) and the acetyl dialdehyde (VII). The structure of the aldehyde (VI) (2,4-dinitrophenylhydrazone: m.p. 136.5~137.5°, MS m/e 336 (M^+)) was established by the following spectral properties: IR bands ($CHCl_3$) at 2840, 2730, 1729 (aldehyde), and 1770 cm^{-1} (γ-lactone), and NMR signals ($CDCl_3$) at 9.81 (t, J=1.5, $C_{(22)}H$), 2.66 (dd, J=1.5, 6, $C_{(23)}H_2$), 2.19 (tdd, J=6, 11, 8, $C_{(24)}H$), 2.36 (dq, J=11, 7, $C_{(25)}H$), 1.26 (d, J=7, $C_{(26)}H_3$), 4.15 (dq, J=8, 6, $C_{(28)}H$), and 1.41 (d, J=6, $C_{(29)}H_3$), mutual couplings being confirmed with the aid of double resonance experiments. The oily dialdehyde (VII), IR bands ($CHCl_3$) at 3440 (hydroxyl), 1728 (aldehyde), 1705 (acetyl), and 1662 cm^{-1} (cyclohexenone), was converted i treatment with silica gel into the crystalline enal (VIII), m.p. 197~200° (decomp.), IR bands (KBr) at 3540 (hydroxyl), 2830, 2730, 1676, 1620 (enal), 1708, and 1700 cm^{-1} (acetyl and cyclohexanone), which was identified as the enal (VIII) obtained from ecdysterone (X) by periodate oxidation followed by silica gel treatment.[11]

The hydroxyls at C-2 and 3 are assigned β-configurations on the basis of the close similarity in the chemical shifts and splitting patterns of the NMR signals for the C-2, 3, and 19 protons of cyasterone and its derivatives to those of the corresponding congeners IX ~ XII and their derivatives (partly shown in Table I. and II.).

Therefore, the structure including some of the stereochemistry of cyasterone is represented by formula I. The configurations at C-20, 22, 24, 25, and 28 remain to be determined and are

now under investigation.

Biological tests with Musca domestica show that the activity of cyasterone is of the same order as that of ecdysterone.

It is of interest to note that, whereas the previously isolated insect-moulting substances are all C_{27} compounds biogenetically related to cholesterol, cyasterone is a C_{29} compound related to sitosterol.

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FOOTNOTES AND REFERENCES

*1 All elemental analyses are consistent with the molecular formulae shown.

*2 NMR spectra were run on Varian HA-100 spectrometers. Chemical shifts are expressed in p.p.m. downfield from internal TMS and coupling constants (J) in c.p.s. Abbreviations: s=singlet, d=doublet, t=triplet, and q=quadruplet.

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