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ISOCYASTERONE, AN INSECT METAMORPHOSING SUBSTANCE FROM *CYATHULA CAPITATA**

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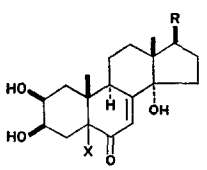
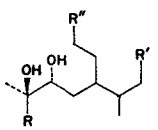
Abstract—A novel C_{29} insect-metamorphosing substance, isocyasterone, has been isolated from *Cyathula capitata* (Amaranthaceae). Chemical and spectroscopic investigations of isocyasterone and its triacetate (II) have shown that isocyasterone has stereostructure I.

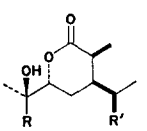
INTRODUCTION

RECENTLY it has become recognized that ecdysterols, active substances causing the moulting and metamorphosis of arthropods, are widely distributed in the plant kingdom.[1] *Inter alia*, we have found that the crude drug Radix Cyathulae, the dried roots of *Cyathula capitata* Moquin-Tandon (Amaranthaceae), utilized as a diuretic or a tonic in the Oriental medicine, contains a number of unique C_{29} phytoecdysones, i.e., amarasterone A (III), amarasterone B (IV),[2] capitasterone (V),[3] precyasterone (VI),[4] cyasterone (VII),[5,6] epicyasterone (VIII),[7] sengosterone (IX)[8] and poststerone (X),[9] these substances being considered to be intermediates in a single synthetic-degradative scheme.

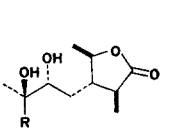




(III) X=5β-H, R'=OH, R''=H
(IV) X=5β-H, R'=H, R''=OH



(V) X=5β-H, R'=H
(VI) X=5β-H, R'=OH



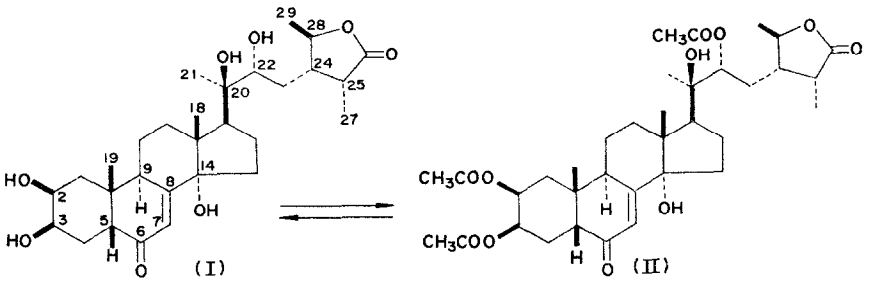
(VII) X=5β-H
(VIII) X=5α-H
(IX) X=5β-OH

(X) X=5β-H

* Part XII in the series "Steroids". For Part XI see H. HIKINO, K. NOMOTO and T. TAKEMOTO, *Steroids* **16**, 393 (1970).

[1] H. HIKINO and Y. HIKINO, *Fortschritte d. Chem. org. Naturst.*, (edited by L. ZECHMEISTER), Vol. 28, p. 256. Springer-Verlag, Wien (1970).
[2] T. TAKEMOTO, K. NOMOTO and H. HIKINO, *Tetrahedron Letters* **1968**, 4953.
[3] T. TAKEMOTO, K NOMOTO, Y. HIKINO and H. HIKINO, *Tetrahedron Letters* **1968**, 4929.
[4] H. HIKINO, K. NOMOTO, R. INO and T. TAKEMOTO, *Chem. Pharm. Bull.* (*Tokyo*) **18**, 1086 (1970).
[5] T. TAKEMOTO, Y. HIKINO, K. NOMOTO and H. HIKINO, *Tetrahedron Letters* **1967**, 3191; H. HIKINO, Y. HIKINO, K. NOMOTO and T. TAKEMOTO, *Tetrahedron* **24**, 4895 (1968).
[6] H. HIKINO, K. NOMOTO and T. TAKEMOTO, *Chem. Pharm. Bull.* (*Tokyo*) **18**, 2132 (1970); *Idem.*, *Tetrahedron* **27**, 315 (1971).
[7] H. HIKINO, K. NOMOTO and T. TAKEMOTO, *Chem. Pharm. Bull.* (*Tokyo*) **19**, 433 (1971).
[8] H. HIKINO, K. NOMOTO and T. TAKEMOTO, *Tetrahedron Letters* **1969**, 1417; *Idem.*, *Tetrahedron* **26**, 887 (1970).
[9] H. HIKINO, K. NOMOTO and T. TAKEMOTO, *Steroids* **16**, 393 (1970).

During the final purification of cyasterone, the main ecdysterol in this crude drug, using repeated crystallization from methanol, a small quantity of a similar substance was noted; although a crude sample showed the same spectral properties and the same TLC behavior as pure cyasterone, TLC of its acetylation product revealed the presence of a minute amount of another acetate along with cyasterone triacetate. Chromatography of the acetate mixture afforded the acetate in question which on alkaline hydrolysis yielded, though in a poor yield, the free ecdysterol. Since this substance is a stereoisomer of cyasterone as will be clarified below, it is now designated as isocyasterone. It is the purpose of this report to present the results leading to the selection of formula I as the representation of the stereostructure of isocyasterone.†



RESULTS

Isocyasterone possesses the molecular formula $C_{29}H_{44}O_8$ as evidenced by the appearance of the peak at *m/e* 502 due to $M-H_2O$ ion in the mass spectrum. Further, the fragmentation pattern is essentially identical with that of cyasterone (VII), suggesting that isocyasterone may have a constitution similar to cyasterone. Also, the UV and IR spectra of isocyasterone and its triacetate (II) are practically the same as those of cyasterone and its 2,3,22-triacetate, respectively, and the NMR spectra of isocyasterone and cyasterone are very similar (Table 1). Thus, the UV maximum at 242 nm (log ε 4·05), the IR band (KBr) at

TABLE 1. METHYL CHEMICAL SHIFTS (PYRIDINE)

		C-18	C-19	C-21	C-27	C-29
Cyasterone	(VII)	1·19	1·06	1 51	1·33 d	1·33 d
Isocyasterone	(I)	1·16	1 04	1·52	1 24 d	1·19 d

1650 cm^{-1}, and the NMR signal at 6·16 ppm shown by isocyasterone indicate the presence of a β,β-disubstituted, α,β-unsaturated ketone group, suggestive of the presence of the 7-en-6-one system. The ORD and CD curves of isocyasterone exhibit negative and positive Cotton effects at 248 and 341 nm for the π-π^* and n-π^* transition of the enone grouping, respectively, which are also consistent with those of cyasterone. These data demonstrate that isocyasterone has a 5β(H)-7-en-6-one moiety. The line positions and splitting patterns of the two signals attributable to two hydrogens on acetoxyl-bearing carbons in the NMR

† A preliminary account of this work has been published: *Chem. Pharm. Bull. (Tokyo)* **19**, 433 (1971).

spectrum of the triacetate (II) coincide closely with those of the C-2 and C-3 hydrogen signals in that of cyasterone triacetate. In the NMR spectrum of isocyasterone or its triacetate (II), the chemical shift of an angular Me proton signal, which is sensitive to both the stereochemistry of and substituents on the steroidal nucleus, is in good agreement with that of the C-19 Me singlet of cyasterone or its triacetate, respectively (Tables 1 and 2).

TABLE 2. PROTON SIGNALS ($CDCl_3$)

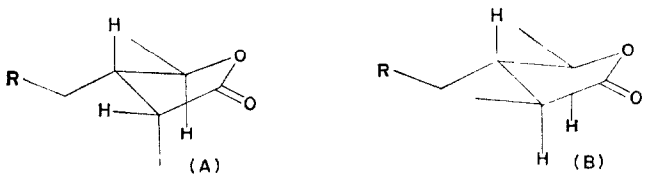
	C-2α	C-3α	C-7	C-9	C-18	C-19	C-21	C-22	C-27	C-28	C-29
Cyasterone 2,3,22-triacetate	~5·01 *	5·31 ddd	5·85 d	3·11 ddd	0·85 s	1·02 s	1·25 s	~4·98 *	1·28 d	4·10 dq	1·41 d
Isocyasterone 2,3,22-triacetate	~5·05 *	5·33 ddd	5·86 d	3·12 ddd	0·85 s	1·02 s	1·28 s	~4·99 *	1·22 d	4·64 dq	1·42 d

* Patterns are unclear.

These observations favor a 2β,3β-dihydroxy structure in isocyasterone. On the other hand, heating of isocyasterone with HCl in methanol displaced the UV maximum affording two new maxima at 297 and 243 nm, a fact which indicates the elimination of a C-14 OH group and the resulting formation of two isomeric dienones, the 7,14-dien-6-one and 8,14-dien-6-one derivatives. Thus, a C-14 hydroxyl group is present in isocyasterone. Further, the chemical shift of another angular Me signal in the NMR spectrum of isocyasterone or its triacetate (II), which agrees well with that of the C-18 Me singlet of cyasterone or its triacetate, respectively along with the previous ORD and CD data, demonstrate the α-configuration of the C-14 OH group. This finding together with the facts that the chemical shift of a proton signal due to a Me on an OH-carrying carbon in the NMR spectrum of isocyasterone or its triacetate (II) is compatible with that of the C-21 Me singlet of cyasterone or its acetate, respectively, and that the position and shape of the signal assigned to a hydrogen on a carbon attached to an acetoxyl group in the spectrum of the acetate (II) is similar to those of the C-22 carbinyl proton signal in that of cyasterone triacetate, indicate the presence of the C-20 and C-22 OH groups which are oriented in the same configurations as those of cyasterone. These structural features were further evidenced by the mass spectrum of isocyasterone which showed the characteristic peaks at *m/e* 363 (M−157, 2% relative to the base peak at *m/e* 44), 345 (M−157−18, 17%) and 327 (M−157−36, 39%), due to the nuclear fragments remaining after loss of the side-chain by the cleavage of the C-20:C-22 bond without rearrangement, which is expected of a vicinal diol.

The side-chain of isocyasterone also has many features in common with that of cyasterone. Thus, the peaks at *m/e* 201 (M−319, 10%) and 183 (M−319−18, 25%), and the peak at *m/e* 157 (M−363, 47%) are the expected side-chain fragments formed by the C-17:C-20 and the C-20:C-22 bond fissions without rearrangement, respectively. Further, the presence of a γ-lactone ring was revealed by an IR band at 1755 cm^{-1}. Analysis of the NMR spectrum of the triacetate (II) with the aid of double resonance experiments indicated that a signal arising from a hydrogen on the carbon carrying the lactonic oxygen was coupled to a secondary Me signal and a hydrogen signal. The somewhat deshielded line position of a Me doublet in the spectrum of isocyasterone or its acetate (II) suggests the presence of a secondary Me group which is adjacent to a CO group. These observations lead to the same gross structure of the side-chain as that of cyasterone.

As has been mentioned above, in the NMR spectra, the chemical shifts of the C-18, C-21 and C-22 protons of isocyasterone and its triacetate (II) are identical with those of cyasterone and its triacetate, respectively, indicating the same 20(*R*), 22(*R*)-configurations, while the chemical shifts of the C-27, C-28 and C-29 protons of isocyasterone and its triacetate (II) are displaced in comparison with those of cyasterone and its triacetate (Tables 1 and 2), suggesting that isocyasterone may be a stereoisomer of cyasterone with respect to some point(s) at C-24, C-25 and C-28. Previously, we have established that part of the side-chain of cyasterone is represented by stereoformula B, the coupling constants

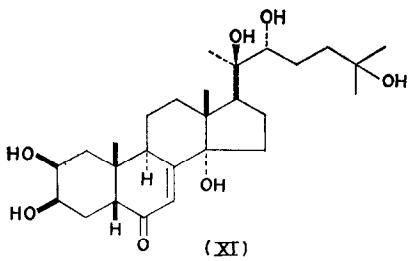


between the C-28 and C-24 diquasiaxial hydrogens and between the C-24 and C-25 diquasiaxial hydrogens being 8 and 11 Hz, respectively.[6] In the NMR spectrum of the triacetate (II), the coupling constants between the C-28 and C-24 hydrogens and between the C-24 and C-25 hydrogens were found to be 7 and 8 Hz, respectively, these values being compatible with a diquasiaxial coupling and quasiaxial-quasiequatorial coupling, respectively. These findings show the *trans* and *cis* relationship of the C-28 and C-24 substituents and the C-24 and C-25 substituents, respectively, as shown in stereoformula A or its enantiomer. If this were the case, the C-25 methyl group and the C-28 hydrogen are to reside in the 1,3-diquasiaxial relationship, this being corroborated by the observation of an intramolecular nuclear Overhauser effect between the C-27 protons and the C-28 proton.

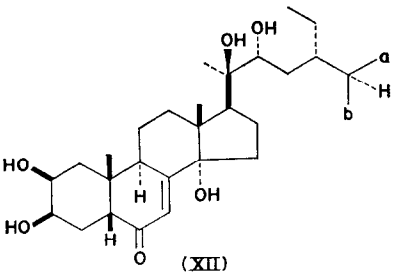
This conclusion was further examined as follows. Since even the acetate (II) is not soluble enough in benzene for an NMR measurement, the solvent-induced shifts caused by passing from chloroform to benzene solution could not be determined. However, the shift from chloroform to pyridine solution ($\delta_{CDCl_3} - \delta_{C_5D_5N}$) for the C-27 Me resonance next to the C-26 lactonic CO group in the acetate (II) is —0·01 ppm, whereas that in cyasterone triacetate is —0·16 ppm. Although there is a tendency for quasiaxial methyls to be shielded more than quasiequatorial methyls in pyridine solution, the observed values are not exactly consistent with those for α-methyls of simple γ-lactones.[6, 10] This discrepancy may be rationalized by effects due to other features of these complexed molecules, e.g., hydroxyl groups associated with pyridine.

Then, the Hudson–Klyne lactone rule was applied to the absolute configuration at C-28 of isocyasterone.[11] First, in order to examine the possibility that addition of alkali causes epimerization at C-5 by the keto-enol tautomerism of the C-6 carbonyl group, which may possibly change the optical rotation, the optical rotations of ecdysterone (XI), which was chosen as the representative of common ecdysterols having no lactone systems, were determined in methanol solution in the absence and presence of alkali. As a result, it was found that addition of alkali does not affect the optical rotation of an ecdysterol if it only possesses the 5β(H)-7-en-6-one system. Next, the optical rotations of isocyasterone

[10] C. R NARAYANAN and N. K VENKATASUHRAMNIAN, *Tetrahedron Letters* **1966**, 5865
[11] C S HUDSON, *J. Am Chem Soc* **32**, 338 (1910); **61**, 1525 (1939); W. KLYNE, *Chem. & Ind* 1198 (1954).



(XI)



(XII)

were measured under the same conditions as used for cyasterone, whose absolute configuration at C-28 has been established as *R*.[6] The data thus obtained showed that addition of alkali made both lactones laevorotatory. Therefore, the absolute configuration at C-28 of isocyasterone was established to be also *R*, the β-configuration of the C-28 methyl group being consequently elucidated. Whereupon it follows that the C-24 and C-25 substituents are both located in the α-disposition.

On the basis of the above evidence, it is concluded that isocyasterone is 25-*epi*-cyasterone as represented by formula I.

It is of interest that the pair of epimers, cyasterone and isocyasterone, occur together in the same plant. Since the inversion of the configuration of the C-27 methyl group from cyasterone to isocyasterone or *vice versa* during the isolation procedure is unlikely, isocyasterone and cyasterone are probably synthesized by the same route; for example, stereochemically unselective hydroxylation at a or b position of the postulated common precursor (XII) would lead to the formation of both cyasterone and isocyasterone.

Isocyasterone was assayed in terms of its ability to provoke puparium formation of isolated larval abdomens of the fleshfly, *Sarcophaga peregrina*. As a result, it was observed that isocyasterone was highly active, though the activity is less than that of cyasterone; the quantitative data will be reported elsewhere.

EXPERIMENTAL

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

Isolation of isocyasterone. 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 (7·5 kg), which on extraction with AcOEt and evaporation gave a residue (170 g). Chromatography of the residue (170 g) over alumina, elution with AcOEt, and crystallization from MeOH furnished cyasterone (VII) as colorless needles (4·0 g). Repeated removal of deposited crystals from the mother liquor left the mixture of cyasterone and isocyasterone as a colorless amorphous mass (40 mg) which was dissolved in Ac_2O (0·2 ml) and pyridine (0·4 ml) and left standing at room temp. overnight. Upon isolation in the usual manner, the product (55 mg) was chromatographed on silica gel (5 g).

Benzene-AcOEt (3:1) eluate (30 mg) was crystallized from MeOH to give cyasterone 2,3,22-triacetate as colorless needles (22 mg), mp 250–252°. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1777 (γ-lactone), 1738, 1235 (acetoxyl), 1658 (cyclohexenone).

Benzene–AcOEt (2:1) eluate (15 mg) was crystallized from MeOH to furnish *isocyasterone 2,3,22-triacetate* (II) as colorless needles (12 mg), mp 255–256°. $[\alpha]_D$ +71 9° (*c* 0·51, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3525, 3475 (OH), 1765 (γ-lactone), 1738, 1238 (acetoxyl), 1659 (cyclohexenone); NMR ($CDCl_3$): 3H s at 0·85 ($C_{(18)}H_3$), 3H s at 1·02 ($C_{(19)}H_3$), 3H d at 1·22 (J = 6, $C_{(27)}H_3$), 3H s at 1·28 ($C_{(21)}H_3$), 3H d at 1·42 (J = 7, $C_{(29)}H_3$), 3H s's at 1·99, 2 10, 2·12 (CH_3COO—), 1H dq at 2·70 (J = 6, 8, $C_{(25)}H$), 1H ddd at 3·12 (J = 2, 8, 10, $C_{(9)}H$), 1H dq at 4·64 (J = 7, 7, $C_{(28)}H$), 1H m at 4·99 ($C_{(22)}H$), 1H m at 5·05 ($C_{(2)}H$), 1H ddd at 5·33 (J = 3, 4, 5, $C_{(3)}H$), 1H d at 5·86 (J = 2, $C_{(7)}H$), NMR (C_5D_5N): 3H s's at 1·06, 1·10 ($C_{(18)}H_3$,

$C_{(19)}H_3$), 3H d at 1·23 ($J = 6$, $C_{(27)}H_3$), 3H d at 1·40 ($J = 7$, $C_{(29)}H_3$), 3H s at 1 56 ($C_{(21)}H_3$), 3H s's at 1·99, 2 05, 2·08 (CH_3COO—), 1H ddd at 3·49 ($J = 2$, 8, 10, $C_{(9)}H$), 1H m at 4·69 ($C_{(28)}H$), 3H m in the range of 5·09 ~ 5·52 ($C_{(2)}H$, $C_{(3)}H$, $C_{(22)}H$), 1H d at 6 14 ($J = 2$, $C_{(7)}H$). (Found: C, 62·99; H, 7·93 $C_{35}H_{50}O_{11}.H_2O$ required: C, 63·23; H, 7·88%.)

To the triacetate (II) (200 mg) in MeOH (5 ml) was added K_2CO_3 (60 mg) in aqueous MeOH (5 ml) and the mixture was set aside at room temp. under N_2 for 1 hr. After isolation in the customary way, the product (140 mg) was chromatographed over silica gel (20 g). Elution with $CHCl_3$–MeOH (20:1) gave *isocyasterone* (I) as a colorless amorphous mass (70 mg). $[\alpha]_D$ +65·3° (*c* 0·487, MeOH); ORD (*c* 0 140, dioxane): $[\phi]_{450}$ +2650, $[\phi]_{375}$ +4480, $[\phi]_{360}$ +4760, $[\phi]_{345}$ +1960, $[\phi]_{333}$ −1270, $[\phi]_{321}$ −2980, $[\phi]_{310}$ −2890, $[\phi]_{285}$ −1490 $[\phi]_{259}$ −6710, $[\phi]_{230}$ +23690, $[\phi]_{210}$ 0; CD (*c* 0 140, dioxane)· $[\theta]_{390}$ 0, $[\theta]_{369}$ +1540, $[\theta]_{355}$ +4000, $[\theta]_{341}$ +4770, $[\theta]_{329}$ +3390, $[\theta]_{295-273}$ 0, $[\theta]_{248}$ −10460, $[\theta]_{234}$ 0, $[\theta]_{224}$ +11080, $[\theta]_{210}$ +4920 UV λ_{max}^{MeOH} nm (log ϵ): 242 (4·05); IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 1755 (γ-lactone), 1650 (cyclohexenone), NMR (C_5D_5N): 3H s at 1·04 ($C_{(19)}H_3$), 3H s at 1·16 ($C_{(18)}H_3$), 3H d at 1·19 ($J = 7$, $C_{(29)}H_3$), 3H d at 1 24 ($J = 6$, $C_{(27)}H_3$), 3H s at 1·52 ($C_{(21)}H_3$), 1H dd at 2 85 ($J = 12$, 4, $C_{(5)}H$), 1H m at 3·45 ($C_{(9)}H$), 1H m at 3·72 ($C_{(22)}H$), 1H m at 4·06 ($C_{(2)}H$), 1H m at 4·10 ($C_{(3)}H$), 1H dq at 4 76 ($J = 7$, 7, $C_{(28)}H$), 1H d at 6·16 ($J = 2$, $C_{(7)}H$).

Acid treatment of isocyasterone. Isocyasterone (0 10 mg) in dil. HCl (conc HCl–MeOH = 1:100, 4 ml) was heated under reflux for 10 min to afford the mixture of the 7,14-dien-6-one and the 8,14-dien-6-one, UV $\lambda_{max}^{MeOH\ (HCl)}$ nm: 297, 243.

Alkali treatment of isocyasterone. To isocyasterone in MeOH was added a trace amount of conc. KOH and left standing at room temp. for 15 min giving the corresponding hydroxy-acid, $[\alpha]_D$ +38·8° (*c* 0 0974, MeOH (KOH)).

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