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STRUCTURE OF AMARASTERONE A AND B, NOVEL C_{29} INSECT-MOULTING SUBSTANCES FROM CYATHULA CAPITATA

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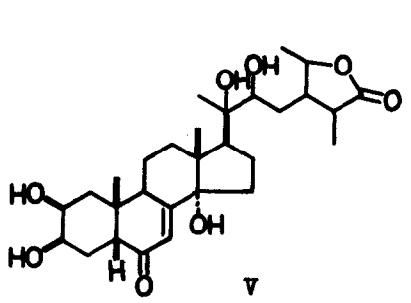
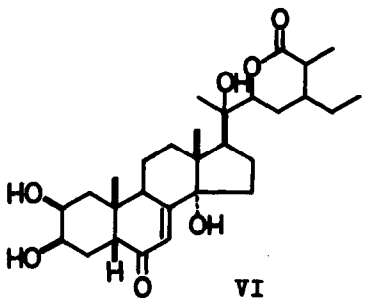
From the roots of Cyathula capitata Moquin-Tandon (Amaranthaceae), the C_{29} insect-moulting substances, cyasterone (V) and capitasterone (VI), have hitherto been isolated.[1,2] Continuation



V

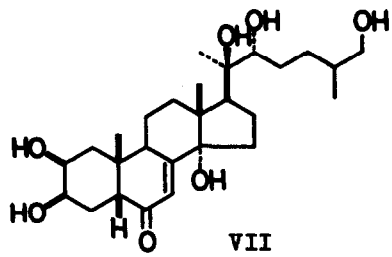


VI

of our work on the analysis of the material has resulted in the isolation of two additional C_{29} ecdysterols which we proposed the names amarasterone A and B. In this communication, we wish to provide evidence that amarasterone A and B are represented by formulas I and II, respectively.

Both amarasterone A, m.p. 210-211°, and amarasterone B, m.p. 284-285°, possess the same composition $C_{29}H_{48}O_7$ (M at m/e 508) and show positive color reactions for steroids. Acetylation of the two steroids gave the tetraacetates (III and IV), m.p. 164.5-165.5° and m.p. 102.5-103.5°, respectively.

The partial structures from C-1 to C-22 of these ecdysterols were concluded to be identical with the partial structure of inokosterone (VII)[3] on the following evidence:[*1] 1) a UV maximum at 244 mμ [244 mμ], an IR band at 1650 cm^{-1} [1650 cm^{-1}], and an NMR signal (1H) at 6.23 p.p.m. [6.22 p.p.m.][*2] (7-en-6-one), 2) treatment with hydrochloric acid in ethanol to give two products which exhibit maxima at 298 and 245 mμ [296 and 244 mμ] (14-hydroxy-7-en-6-one), 3) MS peaks at m/e 363, 345, and 327 [m/e 363, 345, and 327] (nucleus structure and 20,22-dihydroxyls), 4) the C-19 methyl singlet at 1.06 p.p.m. [1.07 p.p.m.] (Table I) (2β,3β-dihydroxyls and 5β-hydrogen),



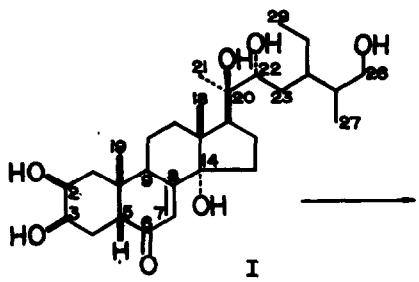
VII

TABLE I. Methyl chemical shifts (pyridine).

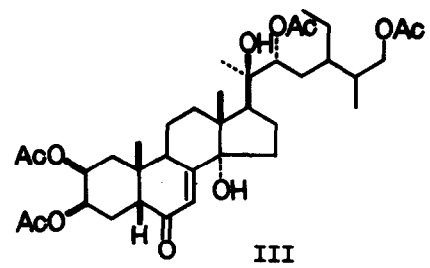
		C-18	C-19	C-21	C-26	C-27	C-29
Inokosterone	(VII)	1.19	1.07	1.52	--	1.03	--
Amarasterone A	(I)	1.21	1.06	1.56	--	1.11d	0.92t
Amarasterone B	(II)	1.22	1.07	1.56	0.91d	0.91d	--

TABLE II. Proton signals ($CDCl_3$).

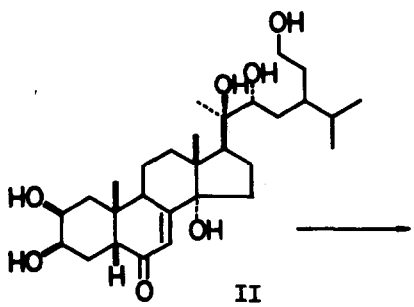
	C-2α	C-3α	C-7	C-9	C-18	C-19	C-21	C-22	C-26	C-27	C-29
Inokosterone 2,3,-22,26-tetraacetate	5.08 ddd	5.35 ddd	5.88 d	3.13 ddd	0.85 s	1.02 s	1.24 s	4.85 dd	3.90 m	0.94 d	--
Amarasterone A 2,3,-22,26-tetraacetate	5.08 ddd	5.34 ddd	5.86 d	3.12 ddd	0.86 s	1.03 s	1.24 s	4.94 dd	3.96 o	0.92 d	0.88 t
Amarasterone B 2,3,-22,29-tetraacetate	5.06 ddd	5.32 ddd	5.86 d	3.10 ddd	0.84 s	1.01 s	1.22 s	4.94 dd	0.84 d	0.86 d	3.92 dd



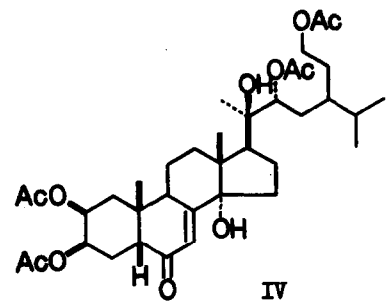
I



III



II



IV

5) the C-2 and C-3 carbinyl hydrogen signals of the tetraacetate (III) [the tetraacetate (IV)], whose line positions and splitting patterns are similar to those of inokosterone tetraacetate (Table II) (2β,3β-dihydroxyls), 6) an ORD Cotton effect, a +75 [a +82], dioxan (5β-hydrogen), 7) the C-18 methyl singlet at 1.21 p.p.m. [1.22 p.p.m.] (Table I) (14α-hydroxyl and 20(R),22(R)-dihydroxyls), and 8) the C-22 carbinyl hydrogen signal of the tetraacetate (III) [the tetraacetate (IV)], of which chemical shift and coupling pattern are consistent with those of inokosterone tetraacetate (Table II) (20(R),22(R)-dihydroxyls).

The IR spectra of amarasterones A and B and those of their tetraacetates (III and IV) are essentially identical, respectively. The MS fragmentation patterns of these two ecdysterols and those of their tetraacetates (III and IV) are also identical, respectively, except for minor differences in relative intensities of certain peaks. These data suggest that both steroids are isomeric.

The mass spectrum of amarasterone A shows characteristic peaks at m/e 145 (M-363), 127 (M-363-18), and 109 (M-363-36) due to the side-chain fragments formed by fission of the C-20:C-22 bond followed by successive elimination of water, indicating that the side-chain fragments are 28 units higher than the corresponding fragments (m/e 117, 99, and 81) in the spectrum of inokosterone (VII). In the NMR spectrum of the tetraacetate (III), the C-22 carbinyl hydrogen signal occurs as a doublet of doublets which demonstrates that the adjacent (C-23) carbon bears two hydrogens. The presence of a CH_3-CH-CH_2-OH moiety was deduced by the observations that the NMR spectrum of the tetraacetate (III) shows a 3H doublet at 0.92 p.p.m. and a 2H octet as an AB portion in an ABX spectrum at 3.96 p.p.m. (δ_A-δ_B= 0.19 p.p.m.), and the doublet and the octet are spin-coupled with a common proton at 1.93 p.p.m. A 3H triplet at 0.92 p.p.m. is clearly visible in the NMR spectrum of amarasterone A, the presence of a CH_3-CH_2- group being shown. All the carbon and hydrogen atoms in the side-chain of amarasterone A have already been settled except for a missing methine group which must connect the -$C_{(23)}H_2$-, the CH_3-CH-CH_2-OH, and the -CH_2-CH_3 group, thus establishing the structure of the side-chain for amarasterone A.

Combined evidence points to the structure of amarasterone A as shown in formula I.

In the NMR spectrum of amarasterone B tetraacetate (IV), the C-22 carbinyl hydrogen signal also appears as a doublet of doublets, a fact which indicates a -CH_2- grouping to be adjacent to C-22. The NMR spectrum of amarasterone B discloses a 6H doublet at 0.91 p.p.m. which was collapsed to a singlet on irradiation at 1.92 p.p.m., demonstrating that a -$CH(CH_3)_2$ system is present. The presence of a >C^*-CH_2-CH_2-OH group in amarasterone B was revealed by a 2H doublet of doublets at 3.92 p.p.m. in the NMR spectrum of the tetraacetate (IV). Combination of the func-

tional groups above deduced, the $-C_{(23)}H_2-$, the $-CH(CH_3)_2$, and the $>C^*-CH_2-CH_2-OH$, leads to the establishment of the side-chain structure of amarasterone B.

Based on the above evidence, it is concluded that amarasterone B has the constitution II.

Amarasterones A and B may be biosynthesized from the hypothetical common precursor, 2β,3β,-14α,20(R),22(R)-pentahydroxy-5β-stigmast-7-en-6-one, by enzymatic hydroxylation at an end of the side-chain (C-26 or C-29). Further metabolism of amarasterone A is most probably carried out in the plant, Cyathula capitata, to give its descendants, capitasterone (VI) and cyasterone (V).

Both amarasterone A and B were revealed to show high moulting hormone activity in the Sarcophaga test.

We are grateful to Analytical Laboratory, Department of Chemistry, this University, for the NMR spectra, and to Naka Works, Hitachi Ltd., for the mass spectra.

FOOTNOTES AND REFERENCES

*1 Data of amarasterone A are presented first and those of amarasterone B are given in [ ].

*2 The NMR spectra of ecdysterols and their acetates are measured on a Varian HA-100 spectrometer in C_5D_5N and $CDCl_3$ solution, respectively. Chemical shifts are expressed in p.p.m. downward from internal TMS. Abbreviations: s=singlet, d=doublet, t=triplet, o=octet, and m=multiplet.

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2) T. Takemoto, K. Nomoto, Y. Hikino, and H. Hikino, Tetrahedron Letters, in press.

3) T. Takemoto, S. Ogawa, and N. Nishimoto, Yakugaku Zasshi, 87, 325, 1474 (1967); T. Takemoto, Y. Hikino, S. Arihara, H. Hikino, S. Ogawa, and N. Nishimoto, Tetrahedron Letters, 1968, 2475.