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STRUCTURE OF SHIDASTERONE, A NOVEL INSECT-MOULTING SUBSTANCE FROM BLECHNUM NIPONICUM

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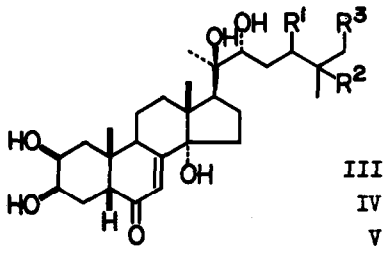
During our survey on the insect-moulting substances in the plant sources, we have undertaken bioassay of the methanol extract of the whole plant of Blechnum niponicum Makino (Blechnaceae) and found that it shows high moulting hormone activity. Chromatography of the polar fraction of the extract has led to the isolation of a new active principle which we name shidasterone. Evidence for its structural assignment as expressed in formula I is presented in this communication.

Shidasterone, m.p. 257-258°, possesses the composition $C_{27}H_{44}O_7$ (M at m/e 480) and gives positive color reactions for steroids. It shows quite similar IR spectrum to the spectra of the common insect-moulting steroids such as ecdysterone (IV), a strong band at 3430 cm^{-1} (hydroxyl) and a characteristic band at 1643 cm^{-1} (cyclohexenone) being visible. The latter band together with a UV maximum at 244 mμ and an NMR signal attributed to a vinyl hydrogen at 6.21 p.p.m.[*1] and ascribable to a 7-en-6-one moiety in the steroid nucleus. When shidasterone was heated in ethanol containing hydrochloric acid, the resultant product showed UV maxima at 298 and 243 mμ. Such behavior is compatible with the 7-en-6-one system having a 14-hydroxyl group.

The NMR spectrum of shidasterone shows the presence of five methyl groups (Table I). Although the observation that the five methyl signals are all singlets agrees with that in ecdysterone (IV), their chemical shifts are not consistent with those of a series of the known phytoecdysones possessing the same molecular formula $C_{27}H_{44}O_7$ (Table I).

The mass spectrum of shidasterone exhibits the fragmentation pattern identical with that of ecdysterone (IV), apart from differences in the relative intensities of certain peaks. In particular, the peaks at m/e 363 (M-117, 29%), 345 (M-117-18, 70%), 327 (M-117-36, 18%), 117 (M-363, 4%), 99 (M-363-18, 100% (base peak)), and 81 (M-363-36, 57%) are associated with the ions formed by the fission of the C-20:C-22 bond and the following dehydration, those characteristic peaks being also present in the spectrum of ecdysterone (IV). This observation shows that two hydroxyl



III: R^1=OH, R^2=R^3=H
IV: R^2=OH, R^1=R^3=H
V: R^3=OH, R^1=R^2=H

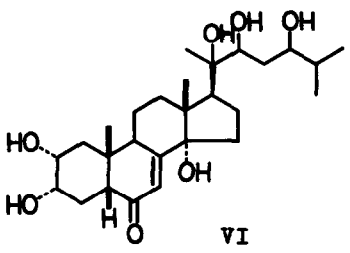


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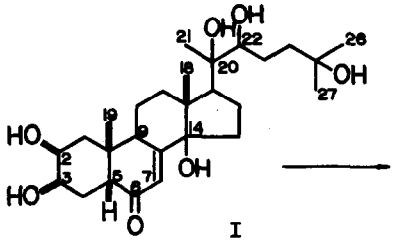
TABLE I. Methyl chemical shifts of ecdysterols ($C_{27}H_{44}O_7$) (pyridine).

		C-18	C-19	C-21	C-26	C-27
Pterosterone	(III)[1]	1.18	1.05	1.54	1.00d	1.00d
Ecdysterone	(IV)[2,3]	1.19	1.04	1.55	1.34	1.34
Inokosterone	(V)[4]	1.19	1.07	1.52	--	1.03d
Ponasterone B	(VI)[5]	1.17	1.11	1.54	0.82d	0.82d
Shidasterone	(I)	1.06	1.06	1.49	1.22	1.22

TABLE II. Proton signals ($CDCl_3$, 100 MHz).

	C-2α	C-3α	C-7	C-9	C-18	C-19	C-21	C-22	C-26	C-27
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
Shidasterone 2,3-diacetate	5.05 ddd	5.32 ddd	5.86 d	3.10 ddd	0.80 s	1.00 s	1.23 s	3.86 dd	1.16 s	1.21 s



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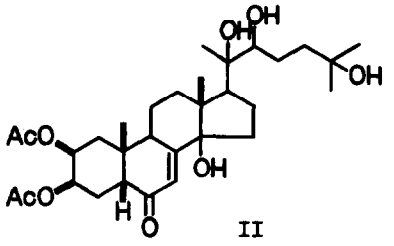

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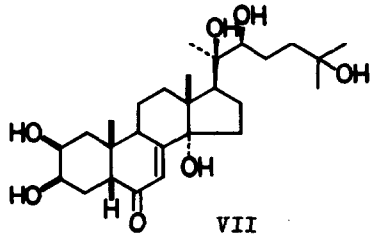

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groups are present at C-20 and C-22 in shidasterone, and that the nucleus and the side-chain of shidasterone are similar to those of ecdysterone (IV). This mass spectral evidence coupled with the previous data leads to postulate that shidasterone may be an isomer of ecdysterone (IV).

The significant difference between shidasterone and ecdysterone (IV) in their chemical properties was that shidasterone gave the 2,3-diacetate (II), m.p. 188~190°, on treatment with acetic anhydride and pyridine at 5°, while ecdysterone (IV) afforded the 2,3,22-triacetate under the same conditions. The following facts indicate the 2β,3β-dihydroxy-5β(H)-stereochemistry for shidasterone: 1) the chemical shifts and splitting patterns of two signals due to two hydrogens on acetoxyl-bearing carbons of shidasterone diacetate (II) are in good accordance with those of the C-2 and C-3 carbinyl hydrogens of ecdysterone triacetate (Table II), 2) the line positions of the C-19 methyl proton signals of shidasterone and its diacetate (II) are compatible with those of ecdysterone (IV) and its triacetate, respectively (Table I and II), and 3) the ORD curve of shidasterone showing a positive Cotton effect centered at 337 mμ ($\underline{a}$ +77) is almost superimposable on the curves of the A/B $\underline{cis}$ substances, $\underline{e.g.}$, ecdysterone (IV). On the other hand, the C-22 hydroxyl group in shidasterone remained unacetylated in the diacetate (II). In consistent with this view, the C-22 carbinyl proton signal appears at 3.87 p.p.m. The splitting pattern of the signal, a doublet of doublets, shows that the adjacent (C-23) carbon bears two hydrogens. Since the side-chain portion contains one more tertiary hydroxyl group, two deshielded methyl singlets at 1.19 and 1.24 p.p.m. demonstrate the presence of a hydroxy-isopropyl moiety. All the carbon and hydrogen atoms in the side-chain have already been accounted for by the above NMR data except for a missing methylene group which consequently must connect the (C-23) methylene and the hydroxy-isopropyl group, thus leading to the deduction of the side-chain structure.

On the basis of the above evidence, it is concluded that the structure together with part of the stereochemistry of shidasterone is represented by formula I. It follows that shidasterone is a stereoisomer of ecdysterone (IV).

Provided that shidasterone, as with ecdysterone (IV), is biosynthesized from cholesterol, and that the biological hydroxylation proceeds with the retention of the configuration, it may be assumed that shidasterone is probably the C-22 epimer of ecdysterone. Recently, we have learned that Schering group has synthesized 22-$\underline{epi}$-ecdysterone (VII).[6] So, direct comparison of shidasterone and 22-$\underline{epi}$-ecdysterone (VII) was performed and, as a result, both substances were revealed to be different. For establishment of the complete stereochemistry of shidasterone, further studies are required.

The insect ($\underline{Sarcophaga}$) bioassay revealed that shidasterone possesses the high moulting hor-

mone activity. It is quite interesting to note that, while 22-epi-ecdysterone shows no activity in the insect test,[6] shidasterone, another stereoisomer of ecdysterone, exhibits high activity. Shidasterone also has the strong stimulating effect on protein synthesis in mouse.

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FOOTNOTE AND REFERENCES

*1 The NMR spectra of the ecdysterol and its acetate are recorded on a Varian HA-100 spectrometer in C_5D_5N and $CDCl_3$ solution, respectively. Chemical shifts are given in p.p.m. downfield from internal TMS. Abbreviations: s=singlet, d=doublet, and dd=doublet of doublets.

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