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STRUCTURE OF PTEROSTERONE

A NOVEL INSECT-MOULTING SUBSTANCE FROM LASTREA THELYPTERIS AND ONOCLEA SENSIBILIS

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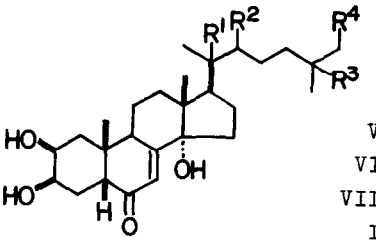
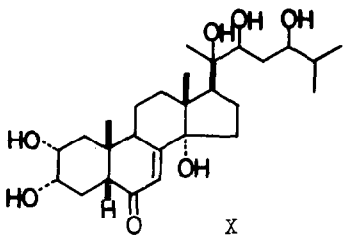
Recently we have reported on the isolation of a novel insect-moulting substance, pterosterone, from Lastrea thelypteris Bory and Onoclea sensibilis Linné (Aspidiaceae).[1] In the present communication, we now wish to propose the structure I for this new steroid.

Pterosterone, $C_{27}H_{44}O_7 \cdot H_2O$,[*1] m.p. 229-230°, $[\alpha]_D$ +7.4° (MeOH), shows a strong band at 3380 cm^{-1} in the IR spectrum (KBr) indicating it to have many hydroxyl groups. In accordance with this view, pterosterone on acetylation afforded the tetraacetate (II), m.p. 116-117°, $[\alpha]_D$ -9.8° ($CHCl_3$), which still exhibits IR absorption (KBr) at 3500 cm^{-1} demonstrating the retention of hydroxyl groups. An α,β-unsaturated carbonyl band at 1641 cm^{-1} in the IR spectrum of pterosterone is consistent with an UV maximum at 243 mμ for a β,β-disubstituted α,β-unsaturated ketone. The UV absorption, after treatment with hot methanolic hydrochloric acid, altered giving two new maxima at 241 and 295 mμ. These observations strongly suggest that pterosterone has the 7-en-6-one chromophore and the 14-hydroxy group in the steroid nucleus as the other known moulting substances (V ~ IX).[2-6]

The mass spectrum of pterosterone is quite similar to that of ecdysterone (VII). The parent peak appears at m/e 480. The characteristic peaks at 462, 444, 426, and 408 are attributed to the loss of one to four molecules of water. The prominent peaks at m/e 363 (M-117), 345 (M-117-18), 328 (M-117-36+1), 327 (M-117-36), 117 (M-363), 99 (M-363-18), and 81 (M-363-36), indicating the same C-20:C-22 side chain cleavage as in ecdysterone, demonstrate that the nucleus of pterosterone is similar to that of ecdysterone, and that the side chain bears three hydroxyl groups, two of which are situated at C-20 and C-22. However, the most significant difference is that the peaks at m/e 99 and 81 are strong in the spectrum of ecdysterone but are weak in that of pterosterone. These data suggest that pterosterone may be either a geometrical isomer with respect to the third hydroxyl group in the side chain or a stereoisomer of ecdysterone.



V: $R^2=R^3=OH$, $R^1=R^4=H$
VI: $R^1=R^2=OH$, $R^3=R^4=H$
VII: $R^1=R^2=R^3=OH$, $R^4=H$
VIII: $R^1=R^2=R^4=OH$, $R^3=H$
IX: $R^1=R^2=R^3=R^4=OH$



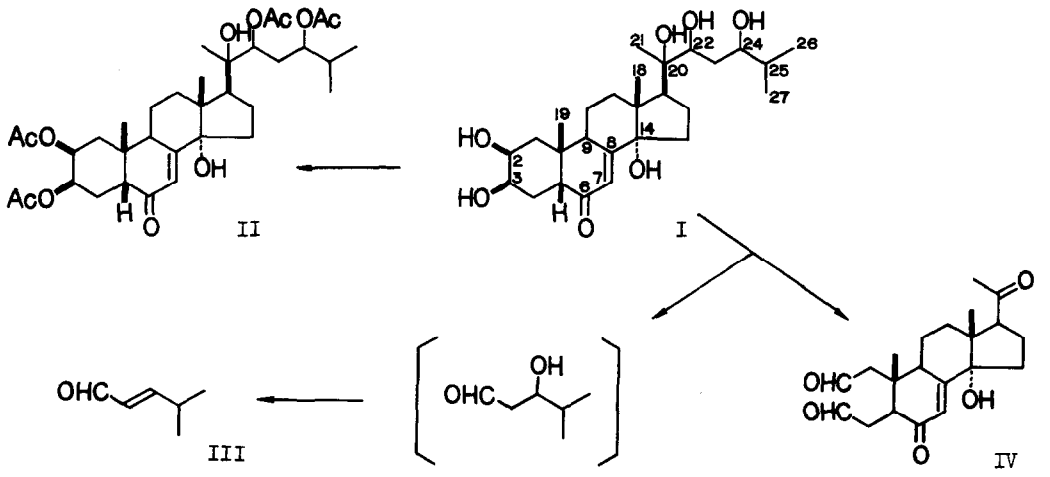
X

TABLE I. Methyl chemical shifts (pyridine).

		C-18	C-19	C-21	C-26	C-27
Ecdysone	(V)[2]	0.74	1.07	1.28d	1.38	1.38
Ponasterone A	(VI)[3]	1.16	1.03	1.51	0.82d	0.82d
Ecdysterone	(VII)[4]	1.19	1.06	1.55	1.34	1.34
Inokosterone	(VIII)[5]	1.19	1.07	1.52	--	1.03d
20,26-Dihydroxyecdysone	(IX)[6]	1.22	1.08	1.58	--	1.48
Pterosterone	(I)	1.18	1.05	1.54	1.00d	1.00d

TABLE II. Proton signals ($CDCl_3$, 100 Mc.p.s.).

	C-2α	C-3α	C-7	C-9	C-18	C-19	C-21	C-22	C-24	C-26	C-27
Ecdysterone 2,3,-22,25-tetraacetate	5.06 ddd	5.33 ddd	5.85 d	3.14 ddd	1.02 s	0.86 s	1.25 s	4.84 dd	--	1.39 s	1.42 s
Pterosterone 2,3,-22,24-tetraacetate	5.07 ddd	5.34 ddd	5.86 d	3.13 ddd	1.02 s	0.85 s	1.23 s	4.91 dd	4.75 ddd	0.90 d	0.90 d



The NMR spectra of pterosterone and ecdysterone are similar (Table I).[*2] Both have singlets assigned to the C-18, C-19, and C-21 methyl protons at the same positions. However, the spectra differ markedly in the positions and patterns of the signals for methyls at the ends of the side chains. Thus, the absence of the six-proton singlet at 1.34 p.p.m. attributed in the spectrum of ecdysterone to the C-26 and C-27 methyls, and instead the presence of a six-proton doublet at 1.00 p.p.m. show that the pterosterone structure has no hydroxyl group at C-25 or C-26. Therefore, the third hydroxyl group in the side chain must be situated at C-23 or C-24. Since it is known that α-hydroxyl groups cause downward shifts of methyl resonances in pyridine solution,[7] the line positions of the C-26 and C-27 methyl signals in pterosterone, which are 0.18 p.p.m. downfield than ponasterone A (VI),[3] suggest the third hydroxyl in the side chain of pterosterone to be oriented at C-24. This arrangement is supported by the fact that in the spectrum of the tetraacetate (II), the signal as a doublet of doublets due to the C-22 carbinyl proton demonstrates the coupling with the adjacent C-23 methylenic hydrogens (Table II); the possibility that the third hydroxyl in the side chain is located at C-23 being excluded.

In order to obtain an unambiguous evidence on the structure of pterosterone, it was oxidized with sodium periodate. The consumption of two molecules of the reagent took place rapidly yielding two fragments originating from the side chain and the nucleus. The side chain fragment was treated with 2,4-dinitrophenylhydrazine in ethanolic hydrochloric acid to afford the 2,4-dinitrophenylhydrazone of the isohexenal (III), m.p. 174~176°. The nucleus fragment was identified as the acetyl dialdehyde (IV) obtained from ecdysterone by periodate oxidation.

Based on the above observations, formula I (exclusive of stereochemistry) is deduced for the structure of pterosterone.

The chemical shifts and splitting patterns of the C-7, 18, 19, and 21 proton signals of pterosterone and of the C-2, 3, 7, 9, 18, 19, and 21 proton signals of its tetraacetate (II) coincide remarkably with those of the corresponding signals of ecdysterone (VII) and other congeners (Table I), and of ecdysterone tetraacetate (Table II), respectively, a fact which indicates that no stereochemical difference in these substances lies within the nucleus environment. This is further confirmed by the fact that the optical rotatory dispersion curve of pterosterone showing the positive Cotton effect (a +69, dioxane) for the $n \rightarrow \pi^*$ transition centered at 339 mμ is almost superimposable on those of the A/B cis substances, e.g., ponasterone A (VI) (a +68, dioxane)[3] and ecdysterone (VII) (a +73, dioxane).[5]

Although it is not yet possible to obtain a conclusive evidence for the stereochemistry in the side chain, the structure along with the absolute configuration in the nucleus of pteroster-

one is thus represented by formula I.

Pterosterone is consequently a stereoisomer of ponasterone C, isolated from Podocarpus nakaii Hayata,[3] which has been recently elucidated to possess the structure X.[8]

In the insect (Sarcophaga) test, pterosterone shows the high moulting hormone activity. Pterosterone, as with the other moulting substances (i.e., ponasterone A, ecdysterone, inokosterone, and cyasterone), exhibits also the high protein anabolic activity of the same order as that of 4-chloro-testosterone, a potent anabolic steroid, in mice.[9]

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FOOTNOTES AND REFERENCES

*1 Analytical data are compatible with the molecular formula shown.

*2 Chemical shifts are expressed in p.p.m. downfield from internal TMS. Abbreviations: s=singlet and d=doublet.

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