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STRUCTURE OF PONASTEROSIDE A, A NOVEL GLYCOSIDE OF INSECT-MOULTING SUBSTANCE FROM PTERIDIUM AQUILINUM VAR. LATIUSCULUM

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We now wish to report the occurrence of a glycoside of an insect-moulting steroid in Nature. This substance was isolated from Pteridium aquilinum Kuhn var. latiusculum Underw. (Pteridiaceae), found to show moulting hormone activity, and named warabisterone.[1] However, since further study has revealed it to be a glycoside, we have changed the term to ponasteroside A after its aglycone ponasterone A (vide infra).

Ponasteroside A, m.p. 278-279.5°, $[\alpha]_D$ +28.5° (pyridine), shows positive color reactions for steroid. The molecular formula was established to be $C_{33}H_{54}O_{11}$ by elemental analysis and the appearance of the molecular ion peak at m/e 626 in the mass spectrum. Its spectral properties are very similar to those of the common insect-moulting substances, e.g., ponasterone A (II).[2-4] Thus the presence of many hydroxyl groups (ν_{max} 3430 cm^{-1}) and a 14-hydroxy-7-en-6-one system in the 5β-steroid nucleus (λ_{max} 245 mμ shifted to 298 and 244 mμ after acid treatment, ν_{max} 1650 cm^{-1}, 1H doublet at 6.18 p.p.m.*, $[\theta]^{max}_{339}$ 45×10^3 (dioxan)) is indicated. The NMR spectrum shows five methyl signals whose chemical shifts and splitting patterns are compatible with those of ponasterone A (II) with the exception that the C-19 methyl proton signal of ponasteroside A is shifted upfield by 0.15 p.p.m. than that of ponasterone A (Table I). At this point it was expected that ponasteroside A is closely related to ponasterone A. However, the composition indicates the presence of $C_6H_{10}O_5$ more than in ponasterone A, and this increment may be corresponding to the linkage of a common hexose. In consistent with this view, the NMR spectrum exhibits that ponasteroside A possesses carbinyl hydrogens much more than ponasterone A. Based on the above observations it was quite reasonable to consider that ponasteroside A might be a glycoside of ponasterone A.

Enzymatic hydrolysis of ponasteroside A was then carried out to give ponasterone A (II), m.p.

TABLE I. Methyl chemical shifts (pyridine).

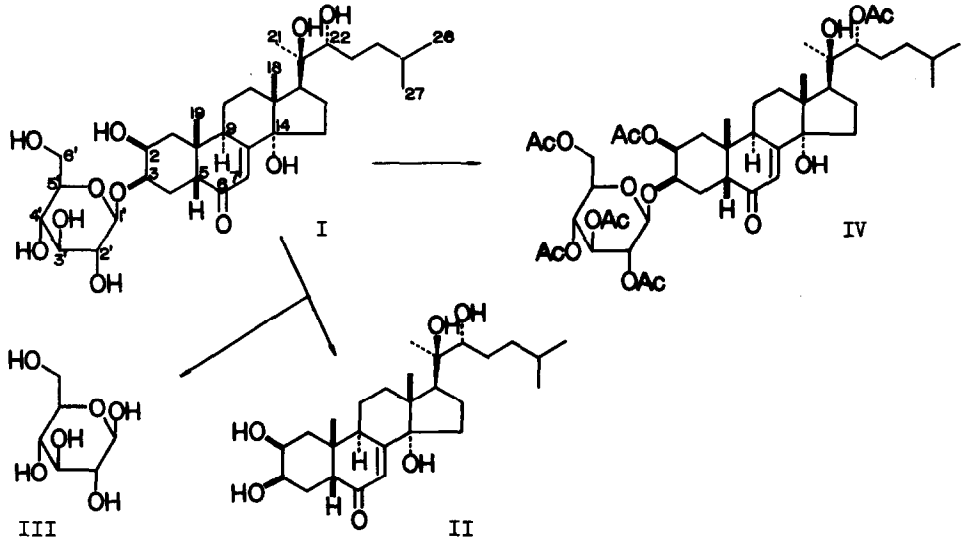
	C-18	C-19	C-21	C-26	C-27
Ponasterone A (II)[2]	1.16	1.03	1.51	0.82d	0.82d
Ponasteroside A (I)	1.17	0.88	1.54	0.84d	0.84d

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
Ponasterone A 2,3,22-triacetate[5]	5.05 ddd	5.32 ddd	5.86 d	3.12 ddd	0.85 s	1.02 s	1.24 s	4.82 dd	0.88 d	0.88 d
Ponasteroside A 2,22,2',3',4',6'-hexaacetate	~4.8 *	~4.15 *	5.82 d	3.05 ddd	0.85 s	0.96 s	1.24 s	~4.8 *	0.88 d	0.88 d

	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'	
Stigmastanyl β-D-glucoside 2',3',4',6'-tetraacetate	4.59 d	4.93 dd	5.22 dd	5.04 dd	3.64 ddd	4.10 dd	4.24 dd
Ponasteroside A 2,22,2',3',4',6'-hexaacetate	4.46 d	~5.02 *	5.22 dd	~5.02 *	3.65 ddd	4.05 dd	4.24 dd

* Patterns are unclear due to overlapping of the signals.



277~278°, and glucose (III), establishing that ponasteroside A is a glucoside of ponasterone A.

As described above, the fact that in the NMR spectrum of ponasteroside A the chemical shift of the C-19 methyl signal is displaced as compared with that of ponasterone A suggests glucose to be linked with ponasterone A at ring A. In order to establish the environment of the glucoside linkage, ponasteroside A was acetylated to give the hexaacetate (IV) whose NMR spectrum was compared with the spectra of ponasterone A triacetate[5] and stigmastanyl β-D-glucoside tetraacetate (Table II). Two signals originating from the two carbinyl hydrogens in ring A of the hexaacetate (IV) appear at ~4.15 and ~4.8 p.p.m. From the line positions, the former signal must be ascribable to a hydrogen on a carbon attached to an oxygen participating the glucoside linkage, and the latter signal attributable to a hydrogen on an acetoxyl-bearing carbon. Although overlapping of the signals does not permit the exact determination of the band width at half height of each signal, the signal shape of the former (~4.15 p.p.m.) indicates that it is associated with an equatorial-like hydrogen, while that of the latter (~4.8 p.p.m.) shows that it is due to an axial-like hydrogen. On the other hand, a chair conformation of ring A of the acetate (IV) was derived from the findings that the ORD curves of ponasteroside A and its acetate (IV) show a similarity to those of ponasterone A (II) and its triacetate, and that an intramolecular nuclear Overhauser effect was observed between the carbinyl hydrogen (~4.8 p.p.m.) and the C-9 allylic hydrogen (3.05 p.p.m.) in the acetate (IV). The combined evidence leads to the conclusion that in ponasteroside A the aglycone is bonded through the C-3 oxygen to glucose.

The C-1' anomeric hydrogen signals in the NMR spectra of ponasteroside A and its acetate (IV) appear as doublets with the large coupling constants (both 7 Hz), demonstrating that glucose is combined in the β-form with the aglycone.

On the basis of the above results, ponasteroside A is concluded to be ponasterone A 3-β-glucoside (I).

Of particular interest biologically is the fact that ponasteroside A, a glycoside, also exhibits high insect-moulting hormone activity in the Sarcophaga test and high stimulating effect on protein synthesis in mouse,[1] though the possibility that ponasteroside A reveals the activities only after enzymatic hydrolysis in animals, cannot be excluded.

We have hitherto been convinced that insect-moulting substances which occur in the plant kingdom must exist partly as glycosides. Ponasteroside A presently characterized constitutes the first example.

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FOOTNOTE AND REFERENCES

* NMR spectra of the ecdysterols and the acetates were recorded on a Varian HA-100 spectrometer in C_5D_5N and $CDCl_3$ solution, respectively. Chemical shifts are given in p.p.m. downward from TMS as internal reference. Abbreviations: s=singlet, d=doublet, and dd=doublet of doublets.

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