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STRUCTURE OF LEMMASTERONE,
A NOVEL C_{29} INSECT-MOULTING SUBSTANCE FROM LEMMAPHYLLUM MICROPHYLLUM

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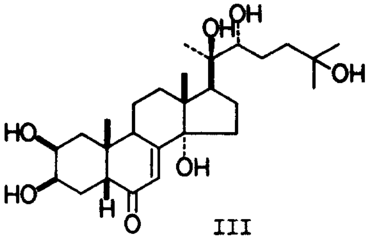
From Lemmaphyllum microphyllum Presl (Polypodiaceae) we have recently isolated, besides pterosterone, ecdysone, and ecdysterone, a novel insect-moulting substance, lemmasterone.[1] In the present communication we now wish to report evidence which leads to the expression I for this new steroid.

Lemmasterone, m.p. 258~259°, shows positive color reactions for steroid and possesses the spectral properties closely resembling those of the other known insect-moulting steroids, *e.g.*, ecdysterone (III).[2~5] The IR spectrum (KBr) exhibits an intense band at 3400 cm^{-1} demonstrating that it contains many hydroxyl groups. The IR absorption at 1643 cm^{-1}, the UV maximum at 244 mμ, and the NMR signal at 6.15 p.p.m.* point to the presence of a β,β-disubstituted α,β-unsaturated ketone system, presumably the same 7-en-6-one chromophore in the steroid nucleus as in ecdysterone (III). The UV maximum, after treatment with hydrochloric acid in ethanol, showed a shift affording two new maxima at 302 and 242 mμ attributed to the 7,14-dien-6-one and 8,14-dien-6-one chromophores, respectively,[6] a fact which suggests that lemmasterone has a C-14 hydroxyl group as well as the 7-en-6-one system. The NMR spectrum exhibits three methyl singlets in similar regions to the C-18, C-19, and C-21 methyl resonances of ecdysterone (III) (Table I). Further two deshielded methyl singlets and a methyl triplet are visible, all of which may be ascribable to the carbon terminals in the side-chain.

The mass spectrum of lemmasterone gave a very weak peak at m/e 508 due to the molecular ion (M). Peaks at m/e 490, 472, 454, and 436 are corresponding to ions produced from M by successive splitting of water, and peaks at m/e 493, 475, 457, and 439 are attributed to those formed from M by the loss of a methyl and further elimination of water. The deduced molecular weight (508) corresponds to the composition $C_{29}H_{48}O_7$ and meets the subsequent spectral and structural requirements. Characteristic peaks at m/e 363 (M-145), 345 (M-145-18), and 327 (M-145-36), also



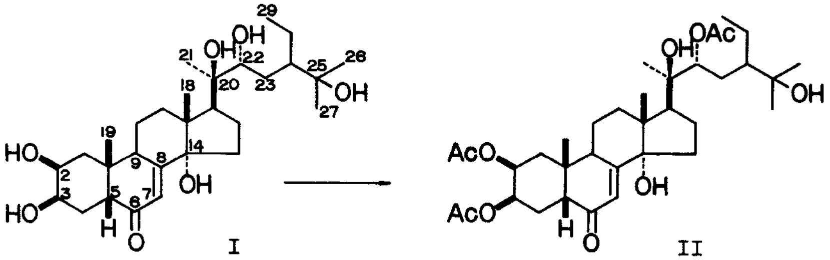
III

TABLE I. Methyl chemical shifts (pyridine).

		C-18	C-19	C-21	C-26	C-27	C-29
Ecdysterone	(III)[3)]	1.19	1.06	1.55	1.34	1.34	--
Lemmasterone	(I)	1.16	1.04	1.50	1.24	1.38	1.04t

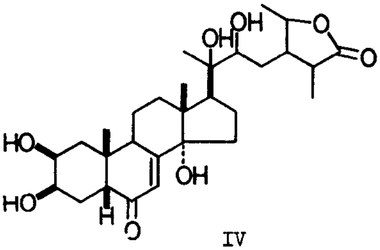
TABLE II. Proton signals ($CDCl_3$).

	C-2α	C-3α	C-7	C-9	C-18	C-19	C-21	C-22	C-26	C-27	C-29
Ecdysterone	5.04	5.31	5.85	3.10	0.85	1.02	1.24	4.79	1.18	1.21	--
2,3,22-triacetate	ddd	ddd	d	ddd	s	s	s	dd	s	s	
masterone	5.06	5.31	5.86	3.10	0.85	1.02	1.24	4.94	1.16	1.19	1.02
2,3,22-triacetate	ddd	ddd	d	ddd	s	s	s	dd	s	s	t



I

II



IV

present in the spectrum of ecdysterone (III), indicate scission of a C-20:C-22 vicinal diol and the following elimination of water, demonstrating that the tetracycles remaining after loss of the side-chains are similar in lemmasterone and ecdysterone (III) and, therefore, the difference between these two substances lies in the side-chains. This interpretation is supported by the fact that in the spectrum of lemmasterone significant peaks originating from the side-chain appear at m/e 145 (M-363), 127 (M-363-18), and 109 (M-363-36), while in that of ecdysterone (III) the corresponding peaks occur at m/e 117 (M-363), 99 (M-363-18), and 81 (M-363-36).

The structure of the side-chain of lemmasterone was elucidated by analysis of the NMR spectrum of its triacetate (II), m.p. 219~220°, prepared by treatment with acetic anhydride in pyridine. The C-22 carbinyl proton signal at 4.94 p.p.m. appears as a doublet of doublets which reveals that the adjacent (C-23) carbon bears two hydrogens. Since the side-chain contains only one more tertiary hydroxyl group, two deshielded methyl singlets at 1.16 and 1.19 p.p.m. indicate the presence of a hydroxy-isopropyl grouping. A methyl triplet at 1.02 p.p.m. shows the presence of an ethyl group. All the carbon and hydrogen atoms in the side-chain have already been accounted for by the above NMR data except for a missing methine group which consequently should connect the (C-23) methylene, the hydroxy-isopropyl, and the ethyl group, thus leading to the establishment of the side-chain structure.

The following NMR spectral properties of lemmasterone and its triacetate support the structure and further deduced some of the stereochemistry. An NMR analysis of the triacetate (II) indicates that the two carbinyl protons at 5.06 and 5.31 p.p.m. are spin-coupled to each other and further coupled with different methylene protons, demonstrating that lemmasterone has a $-CH_2-CH(OH)-CH(OH)-CH_2-$ system which can only be located at the C-1~C-4 position. The coincidence of the chemical shifts of the C-19 proton signals in lemmasterone and ecdysterone (III), and also the similarity of the chemical shifts and splitting patterns of the C-2, C-3, and C-19 proton signals in the triacetate (II) and ecdysterone triacetate (Table I and II) suggest a 2β,-3β-dihydroxy-5β-H structure for lemmasterone. This assignment was confirmed by the ORD curve of lemmasterone which is almost superimposable on that of ecdysterone (III). The C-18 and C-21 methyl chemical shifts of lemmasterone and its triacetate (II), which are in agreement with those of ecdysterone (III) and its triacetate, respectively (Table I and II), show that the configurations at C-14, C-17, C-20, and C-22 are the same in both substances. The displacement of the C-22 proton signal (4.94 p.p.m.) of the acetate (II) as compared with that (4.79 p.p.m.) of ecdysterone triacetate may be due to the effect of the C-24 ethyl group.

On the basis of the above evidence, it is concluded that the structure together with part of the stereochemistry of lemmasterone is expressed by formula I.

In the insect (Sarcophaga) test, lemmasterone exhibits the high moulting hormone activity.[1] Lemmasterone, as with the other insect-moulting substances,[7] shows also the high stimulating effect on protein synthesis in mouse liver.[1]

Lemmasterone has now proved to be the second example of insect-moulting substances possessing 29 carbon atoms, the first C_{29} moulting substance being cyasterone (IV) isolated from Cyathula capitata Moquin-Tandon (Amaranthaceae).[8]

We thank Analytical Laboratory, Department of Chemistry, this University, for the NMR spectra, and Research Laboratories, Takeda Chemical Industries, Ltd., for the mass spectrum.

FOOTNOTE AND REFERENCES

* The NMR spectra of lemmasterone and its acetate 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. downfield from internal TMS. Abbreviations: s=singlet, d=doublet, and t=triplet.

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ADDENDUM

Immediately before the submission of this paper, we have learned from Prof. Nakanishi, this University, that Dr. Horn, CSIRO, and the Takeda group have isolated similar compounds, podecdysone and makisterone C, from Podocarpus elatus R. Brown and P. macrophyllus Lambert (Podocarpaceae), respectively, and in their independent works arrived at the same conclusion regarding the structures as our own. A detailed direct comparison of these substances will be made at a later stage.